Gene expression of miRNA-138 and cyclin D1 in oral lichen planus

Noha A. Ghallab1 · Rehab Fawzy Kasem2 · Safa Fathy Abd El-Ghani2 · Olfat G. Shaker3

Abstract
Objectives This study aimed to evaluate microRNA-138 (miR-138) gene expression and its target cyclin D1 (CCND1) gene and protein expression in oral lichen planus (OLP) mucosa in an attempt to investigate their possible roles in OLP immunopathogenesis.

Methods Sixty oral biopsy specimens were harvested from 30 healthy subjects and 30 OLP patients, subdivided into reticular, atrophic, and erosive groups (n = 10 each). Samples were subjected to quantitative real-time polymerase chain reaction analysis for quantification of miR-138 and CCND1 relative gene expression and immunohistochemical analysis to determine CCND1 protein expression.

Results Samples from OLP patients had a significant underexpression of miR-138 gene and overexpression of CCND1 at both gene and protein levels compared to normal mucosa samples. The lowest levels of miR-138 expression were observed in atrophic and erosive OLP compared to reticular OLP, and the highest levels of CCND1 gene and protein expression were in atrophic OLP. An inverse correlation was demonstrated between the miR-138 expression and both CCND1 gene and protein expression in OLP patients. A significant positive correlation between CCND1 gene and protein expression was also observed.

Conclusion Downregulation of miR-138 increases the gene and protein expression of its potential target CCND1 in OLP mucosa which might have a pivotal role in the disease pathogenesis.

Clinical relevance This research implied that miR-138 may have a role in identification of symptomatic OLP lesions. MiR-138 might be considered as a potential tool in future OLP molecular therapy.

Keywords miRNA-138 · Cyclin D1 · Oral lichen planus · Premalignant lesion · Tumor suppressor

Introduction
Oral lichen planus (OLP) is a relatively common chronic inflammatory mucocutaneous disease. It affects 1–2% of the adult population, women more than men, mainly middle aged and elderly [1, 2]. Clinical presentation of OLP ranges from asymptomatic reticular white striae to symptomatic atrophic-erosive red lesions with symptoms of burning, irritation, and pain. To date, OLP is regarded as a T cell-mediated disease; however, the precise pathogenesis remains obscure [3, 4]. OLP is classified as a potentially malignant disorder by the World Health Organization (WHO) with a highly variable risk of developing into head and neck squamous cell carcinoma (HNSCC) (0.4–12.5%) [5–7]. Currently, there are few prognostic markers to identify which chronic OLP lesions are at a higher risk for progression. Therefore, it is extremely urgent to unravel the underlying molecular mechanisms of malignant transformation of OLP so that accurate diagnosis can be made and new therapeutic approaches can be developed [8].

Recent studies revealed an important role of microRNAs (miRNAs) in the pathogenesis of OLP [8, 9]. MiRNAs are
endogenous small noncoding 22-nucleotide-long RNA molecules that control the target gene expression at the posttranscriptional level [10, 11]. Each miRNA can control a large number of target mRNAs and each mRNA can be controlled by many miRNAs [12, 13]. MiRNAs participate in the regulation of diverse cellular processes including proliferation, differentiation, development, and apoptosis [11, 14]. Recently, alterations in miRNA expression were reported to be involved in many physiological and pathological processes, including chronic inflammatory, autoimmune diseases [15, 16] and HNSCC [17–21]. Moreover, studies suggested that there is a particular miRNA signature associated with the malignant progression of oral premalignant lesions [22–24].

MiRNAs are functionally integrated into many crucial cell cycle control pathways [25]. MicroRNA-138 (miR-138) is a multifunctional molecule regulator that regulates a variety of biological processes [26]. Studies reported that the downregulation of miR-138 was involved in the pathogenesis of different diseases [27, 28]. While deregulation of miR-138 has been frequently observed in HNSCC [29–32], the precise role of miR-138 in tumorigenesis is still elusive. Moreover, Liu et al. [33] identified the cyclin D1 (CCND1) gene as a novel direct target of miR-138 using bioinformatic estimation. CCND1 is a proto-oncogenic positive regulator of the cell cycle, driving cells from G1 into S phase checkpoint [34]. It is a 45-kD protein encoded by CCND1 gene located on chromosome 11q13 [35]. Previous reports suggested that CCND1 might have a role in the pathogenesis of OLP [36–38]. In addition, other studies reported alterations in the expression of cell cycle regulatory proteins in oral premalignant and malignant lesions [35, 39–41]. Nonetheless, recognizing the regulatory networks underlying the altered expression of these genes in the development of OLP is far from complete. Given that miRNAs appear to constitute one of the largest classes of gene regulatory molecules, understanding their mode of action and their pathological roles is essential [42].

Although OLP is currently discussed as a status with malignant potential, the premalignant potential of OLP and how it develops into oral cancer are not fully elucidated [43, 44]. In light of the regulatory properties of miRNAs and its impact on tumor development, there is an urgent and great need to explore the molecular mechanisms underlying the pathogenesis of OLP, indicating new therapeutic targets. Based on the authors’ knowledge, no previous studies in the literature have investigated the target relationship between miRNA-138 and CCND1 expression in OLP mucosal tissue. Accordingly, the aim of the present investigation was to evaluate the expression of miR-138 and its target CCND1 protein and gene expression in OLP lesions compared to healthy controls utilizing quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry. This study was conducted in an attempt to investigate the possible role of miR-138 on the regulation of CCND1 in OLP immunopathogenesis.

Materials and methods

This clinical trial has been registered at ClinicalTrials.gov (identifier NCT02834520).

Study population

The entire study sample comprised 60 subjects: 30 patients suffering from OLP (19 females, 11 males; their age ranging from 46 to 69 years) and 30 age-sex matched control subjects (19 females, 11 males; their age ranging from 45 to 65 years) who were healthy normal individuals free from any inflammatory oral lesions.

Inclusion and exclusion criteria

All individuals enrolled in this study were selected from the Outpatient Clinic, Department of Oral Medicine, Periodontology and Diagnosis, Faculty of Oral and Dental Medicine, Cairo University during the period from May 2015 to December 2015. A detailed medical history of each subject was obtained according to the detailed questionnaire of the modified Cornell Medical Index [45]. To qualify for the study, patients were diagnosed with OLP according to the WHO’s clinicopathological diagnostic criteria for OLP [46]. According to the clinical features, the OLP patients were divided into three subgroups, including 10 reticular OLP, 10 erosive OLP, and 10 atrophic OLP cases. The presence of Wickham’s striae confirmed the clinical diagnosis. These striae got accentuated by stretching of the surface mucosa and were not eliminated by rubbing. The oral lesions were bilateral and extended to involve the buccal mucosa, labial mucosa, and tongue which varied from one patient to the other. Duration of the disease ranged from 5 to 6 months with periods of remission and exacerbation. Exclusion criteria included patients with suspected restoration-related reaction or under any medication that could cause lichenoid reaction during the 3 months before the study, gingival inflammation, any systemic disease, inflammatory oral lesions, immunodeficiency, autoimmune disorders, hepatitis, human immunodeficiency virus infection, pregnancy or lactation, and former or current smokers. Patients did not use any topical or systemic medications for treatment of OLP in the last 3 months.

Following an explanation of the study as well as the information about the sampling procedures, each subject signed a written informed consent form approved by the Faculty Research Ethics Committee (September 2014). After obtaining patients’ written consent, 60 tissue biopsy specimens were harvested from representative lesions clinically diagnosed as OLP (n = 30) and from healthy normal oral mucosa as control (n = 30) under local anesthesia with 2% xylocaine adrenaline. All tissue specimens were processed by the Department of Oral Pathology, Faculty of Oral and Dental...
Medicine, Cairo University. Analyses were performed on coded samples by one of the authors (KR), who was masked with regard to the subjects’ diagnoses until all analysis were finished. The histopathologic diagnosis and examination of all specimens were confirmed by two oral pathologists (FS, KR).

**Tissue specimens**

A total of fresh 60 specimens were transformed into paraffin-impregnated tissue blocks including normal oral mucosa as a control group (n = 30) and OLP (n = 30; 10 reticular OLP, 10 atrophic OLP, and 10 erosive OLP). Thin (5 µm) paraffin sections of each tissue specimen were stained with H&E to reconfirm the diagnosis. Other 4-µm paraffin sections of each specimen were mounted on positively charged glass slides (Optiplus; BioGenex, Milmont Drive, CA, USA) for immunostaining with anti-cyclin D1 antibody. Ten sections of 5-µm thickness were cut from each sample and placed in plastic Eppendorf tubes to be subjected equally to a qRT-PCR analysis for miR-138 and cyclin D1 gene expression.

**Immunohistochemistry**

The 60 paraffin-embedded tissue sections on positively charged slides were immunostained with anti-cyclin D1 antibody with super sensitive biotin–streptavidin staining technique. Tissue sections were deparaffinized, rehydrated, and treated with endogenous peroxidase in 0.3% H2O2 for 30 min to block the endogenous peroxidase activity. For antigen retrieval, the slides were boiled in 10 mM citrate buffer, pH 6.0, for 10–20 min followed by cooling at room temperature for 20 m. The positive test slides were incubated with the primary antibody rabbit polyclonal antibody anti-cyclin D1 antibody (Cat. no. RB-9041-R7) Thermo Scientific, Labvision, Kalamazoo, MI, USA) for 30 min at room temperature in a humified chamber. On the other hand, the negative control slides were not exposed to the primary antibody. After washing with phosphate buffer solution (PBS), the slides were treated with the biotin-labeled link antibody for 30 min, then the streptavidin-conjugated horseradish peroxidase was used. The diaminobenzidine chromogen was applied to visualize the antigen–antibody reaction. All these reagents belong to the universal Labeled Streptavidin-Biotin 2 System, Horseradish Peroxidase (code no. K0673 DakoCytomation, Glostrup, Denmark). All the slides were immersed in Mayer’s hematoxylin for counterstaining. Finally, the sections were covered by coverslips using aqueous mounting medium.

The ordinary light microscope was used to detect and localize the immunostaining of anti-cyclin D1 antibody. Cells with nuclear staining were considered positive. The number of cyclin D1-positive cells in the basal and parabasal layers of control and OLP cases was counted. The parabasal layers were defined as the second and third rows above basement membrane of stratified squamous epithelium. At least 500 basal and parabasal cells were counted. The cyclin D1 labeling index was expressed as the number of positive cells per total number of cells in the basal and parabasal layers [47].

**MicroRNA extraction**

MicroRNAs was extracted from paraffin block sections by miRNeasy extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The extracted microRNA was stored at −80 °C until use.

**qRT-PCR analysis for miR-138 expression**

Reverse transcription was carried out on extracted microRNA in a final volume of 20-µL RT reactions using the miScript II RT kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. qRT-PCR was performed using a miScript SYBR Green PCR kit (Qiagen, Valencia, CA,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic and clinical data of subjects</th>
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<tr>
<td>Clinical characteristics of subjects</td>
<td>Mean (SD) of age (year)</td>
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<tr>
<td>Control group (n = 30)</td>
<td>58.77 (5.47)</td>
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<tr>
<td>Oral lichen planus group (n = 30)</td>
<td>60.73 (6.0)</td>
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<tr>
<td>Reticular OLP (n = 10)</td>
<td>59.33 (5.02)</td>
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<tr>
<td>Erosive OLP (n = 10)</td>
<td>60.5 (2.55)</td>
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<tr>
<td>Atrophic OLP (n = 10)</td>
<td>63.7 (7.15)</td>
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<tr>
<td>Localization of OLP lesion</td>
<td></td>
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<tr>
<td>Buccal mucosa</td>
<td></td>
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<tr>
<td>Labial mucosa</td>
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<td>Tongue</td>
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<td>Gingiva</td>
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<td>Palate</td>
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USA) and miScript primer assay miR-138 (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions using Rotor-gene Q Real-time PCR system (Qiagen, USA). After the PCR cycles, melting curve analyses were performed to validate the specific generation of the expected PCR product. SNORD 68 was used as an endogenous control. The expression level of miR-138 was evaluated using the ΔCt method. The cycle threshold (Ct) value is the number of quantitative PCR cycles required for the fluorescent signal to cross a specified threshold. ΔCt was calculated by subtracting the Ct values of SNORD 68 from those of target microRNA. ΔΔCt was calculated by subtracting the ΔCt of the control samples from the ΔCt of the disease samples. The relative gene expression of miR-138 was calculated by the equation $2^{-\Delta\Delta C_t}$ [48].

qRT-PCR analysis for cyclin D1 expression

The commercial QIAamp RNA Mini Kit (Qiagen, USA) was used for RNA extraction. The RNA integrity and concentration were determined by NanoDrop measurement at 260 nm. One microgram of extracted RNA was reverse transcribed to cDNA with high cDNA Reverse Transcriptase Kit. cDNA was amplified for the expression of cyclin D1 and β-actin with SYBR green fluorophore following the manufacturer’s recommended amplification procedure. The sequences of primers used for real-time PCR analysis were as follows: cyclin D1 forward 5′-CGGAGGACAACAAACAGATC-3′ and reverse 5′-GGGTGTGCAAGCCAGGTCCA-3′ and β-actin forward primer 5′-AACCCGAGAAGATGACCGATCATGTTT-3′ and reverse 5′-AGCAGCGTCGTCCTCAGGGCA TCTCTTGCTCAAGTC-3′. The relative quantification of cyclin D1 gene was determined using the comparative CT method. The ΔCt was calculated as the difference between the average Ct values of the β-actin from the average Ct value of cyclin D1 gene. The ΔΔCt was determined by subtracting the ΔCt of the control from the ΔCt of the OLP. Samples were run in triplicate. Relative expression of the target gene was calculated by the equation $2^{-\Delta\Delta C_t}$ [48] which was the amount of cyclin D1 product, normalized to the endogenous control (β-actin) and relative to the control sample.

Statistical and power analysis

Using G-power analysis program [49], sample size was determined by comparing cyclin D1 index in the control and OLP groups according to Hirota et al. [47]. A total sample size of 44 patients was calculated to be sufficient to detect effect size ($f = 1.15$) by considering level of significance α = 0.05, with 95% power. This number was increased to 60 patients (30 in each group) to increase the validity of the results. Data were presented as mean ± standard deviation (SD) or median (min–max). Mann-Whitney U test was used for pairwise comparisons when Kruskal-Wallis test was significant, and for correlation analyses, Spearman’s rank correlation coefficient was performed. Results were displayed by the use of box plots, with the rectangle representing 50% of the cases and the whiskers going out to the smallest and largest value. The median value is displayed by the line inside the rectangle. All tests were two sided, and $P$ values ≤0.05 were accepted for statistical significance. All data were processed with a computerized statistical package (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL).

![Photomicrograph of normal oral mucosa showing negative CCND1 immunoexpression of anti-cyclin D1 antibody (×400).](http://www.clinoralinvest.com)

![Photomicrographs of reticular OLP showing CCND1 positivity of anti-cyclin D1 antibody (×200 and ×400).](http://www.clinoralinvest.com)

![Erosive OLP showing CCND1 positivity of anti-cyclin D1 antibody (×200 and ×400).](http://www.clinoralinvest.com)

![Atrophic OLP showing CCND1 positivity of anti-cyclin D1 antibody (×200 and ×400).](http://www.clinoralinvest.com)

![CCND1-positive basal cells (arrows) of anti-cyclin D1 antibody ×400](http://www.clinoralinvest.com)
**Results**

Table 1 shows demographic data for all subjects included in this study.

**Immunohistochemical detection of cyclin D1 protein in OLP**

Evaluation of CCND1 protein expression occurred by the use of immunohistochemistry performed by one of the investigators (KR). In normal oral epithelium \((n = 30)\), no expression of CCND1 protein was seen (immunonegative). In all specimens \((n = 30)\) of OLP (100%), CCND1 protein was expressed prominently compared with those in the control mucosa. The positivity of CCND1 was detected as a dense nuclear stain which was concentrated at the basal and the parabasal cell layers. The subepithelial connective tissue zone which is just below the epithelium contains dense band of lymphocytes (Fig. 1). Statistical analysis of the CCND1 labeling index revealed that CCND1 protein was significantly highly expressed in atrophic OLP cases compared to reticular OLP \((P < 0.05)\). Although erosive OLP cases also showed high CCND1 protein expression, yet it was not significant when compared to both reticular OLP \((P = 0.96)\) and atrophic OLP groups \((P = 0.242)\) (Table 2).

**Relative gene expression of miRNA-138 in OLP by qRT-PCR**

miRNA-138 relative gene expression was successfully detected in all 60 samples by qRT-PCR. Results showed significantly lower expression of miR-138 \((P < 0.001)\) in OLP samples compared to normal oral mucosa (Fig. 2a). No differences in miRNA-138 expression in OLP epithelium based on sex or age could be detected. The least miRNA-138 expression was observed in atrophic OLP group followed by erosive OLP and reticular OLP groups. Moreover, subgroup analysis revealed that miRNA-138 expression was significantly higher \((P < 0.01)\) in reticular OLP compared to both atrophic and erosive OLP; however, no significant difference \((P = 0.968)\) was detected between atrophic OLP and erosive OLP subgroups (Table 2). In addition, the fold change regarding miR-138 was approximately 0.4-fold in OLP lesions compared to normal tissue. In subgroup analysis, miR-138-fold change in reticular, atrophic, and erosive OLP samples was approximately 0.8-, 0.5-, and 0.2-fold compared to normal controls, respectively.

**Spearman’s rank correlation coefficient analysis**

In OLP tissue, a statistically significant negative correlation was detected between the miR-138 relative gene expression levels and its potential target CCND1 at both the protein level \((q = -0.417, n = 30, P < 0.05)\) and mRNA gene level \((q = -0.483, n = 30, P < 0.001)\) (Figs. 3 and 4, respectively). Furthermore, statistical analysis showed a statistically significant strong positive correlation between CCND1 relative gene expression and CCND1 protein expression \((q = 0.754, n = 30, P < 0.001)\) in OLP tissue (Fig. 5).

**Table 2** Relative gene expression of miRNA-138 and cyclin D1 and cyclin D1 index in all studied groups

<table>
<thead>
<tr>
<th></th>
<th>miRNA-138 (relative gene expression)</th>
<th>Cyclin D1 (relative gene expression)</th>
<th>Cyclin D1 index (protein expression)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Min–max</td>
<td>Median</td>
</tr>
<tr>
<td>Control group ((n = 30))</td>
<td>4.6</td>
<td>3.1–6.88</td>
<td>1.24</td>
</tr>
<tr>
<td>Oral lichen planus group ((n = 30))</td>
<td>2.05(^a)</td>
<td>0.11–4.32</td>
<td>7.74(^a)</td>
</tr>
<tr>
<td>Reticular OLP group ((n = 10))</td>
<td>3.38</td>
<td>1.03–4.3</td>
<td>4.74</td>
</tr>
<tr>
<td>Erosive OLP group ((n = 10))</td>
<td>1.82(^a)</td>
<td>0.11–2.5</td>
<td>9.88</td>
</tr>
<tr>
<td>Atrophic OLP group ((n = 10))</td>
<td>1.05(^b)</td>
<td>0.5–4.32</td>
<td>11.13</td>
</tr>
</tbody>
</table>

\(^{a}\) Statistically significant when compared to control \((P < 0.001)\)

\(^{b}\) Statistically significant when compared to reticular OLP \((P < 0.05)\)
**Discussion**

The comprehension of etiology and pathogenesis of OLP is one of the major challenges in oral pathology and oral medicine. miRNAs are increasingly being recognized as critical regulators of tissue-specific patterns of gene expression [50]. Based on the authors’ knowledge, this study revealed for the first time a significant lower miR-138 gene expression and higher CCND1 gene and protein expression in OLP patients compared to normal oral epithelium using qRT-PCR and immunohistochemical analysis.

The present immunohistochemical findings were similar to those reported by Hirota et al. [47], who suggested that the significant increase in CCND1 index could induce the proliferation status of OLP epithelium. As explained by the authors, in the basal and parabasal cell layers of OLP mucosa, most injured cells enter the cell cycle for proliferation and repair, while the remaining cells undergo apoptosis due to severe DNA damage. In this context, cell cycle arrest and
upregulation of proliferation activity are concomitantly seen, and these contradictory changes could contribute to the development of characteristic mucosal architecture and clinical manifestations of OLP [47] which supports the current observations. The present results were also consistent with other reports suggesting that CCND1 plays an important role in the occurrence and development of OLP [36–38]. This study showed positive CCND1 expression in 100% of OLP cases, which is in line with Abid and Merza [38], Zhang et al. [37], and Yao et al. [36] who reported CCND1-positive expression in 84%, 71.67%, and 82% of OLP cases, respectively. In agreement with the abovementioned studies, these data suggest that the currently observed increase of CCND1 at both protein and mRNA levels in OLP may lead to increased cellular proliferation and might denote the hyperproliferative status of epithelial cells. Other studies reported the proliferation activity of epithelial cells in OLP showing alterations of the cell cycle regulatory mechanism [51, 52]. Although the mechanism of enhanced cell proliferation in OLP remains unsolved, Gonzalez-Moles et al. [53] proposed that this hyperproliferative status in OLP is a compensatory mechanism of epithelium to maintain its architecture in spite of aggressive lymphocyte attack. In addition, Rezaee et al. [52] suggested that cyclin overexpression noted in OLP caused increased cell proliferation due to shortening in the cell cycle G1 phase.

The lower expression of miR-138 in OLP tissue demonstrated in this study was consistent with numerous studies which proposed that alterations in miRNAs expression may play a critical role in the pathogenesis of OLP [50, 54–58]. These studies suggested that the discovery of miRNAs is a new way to unveil the molecular mechanisms underlying OLP and might represent a novel candidate biomarker for diagnosis [8, 9] which supports the current data. Although it is not possible to identify the exact role of miR-138 in OLP, some hypothesis could be drawn. Recent reports provided explanation

### Table 3 Delta Ct (ΔCt) of miRNA-138 and cyclin D1 in all studied groups

<table>
<thead>
<tr>
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<th>ΔCt miRNA-138</th>
<th>ΔCt Cyclin D1</th>
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<tr>
<td></td>
<td>Median</td>
<td>Min-max</td>
</tr>
<tr>
<td>Control group (n = 30)</td>
<td>−2.2</td>
<td>−1.63 to −2.78</td>
</tr>
<tr>
<td>Oral lichen planus group (n = 30)</td>
<td>−1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.184 to −2.11</td>
</tr>
<tr>
<td>Reticular OLP group (n = 10)</td>
<td>−1.76</td>
<td>−0.04 to −2.1</td>
</tr>
<tr>
<td>Erosive OLP group (n = 10)</td>
<td>−0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.18 to −1.32</td>
</tr>
<tr>
<td>Atrophic OLP group (n = 10)</td>
<td>−0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 to −2.11</td>
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</table>

<sup>a</sup> Statistically significant when compared to control (P < 0.001)

<sup>b</sup> Statistically significant when compared to reticular OLP (P < 0.05)

The lower expression of miR-138 in OLP tissue demonstrated in this study was consistent with numerous studies which proposed that alterations in miRNAs expression may play a critical role in the pathogenesis of OLP [50, 54–58]. These studies suggested that the discovery of miRNAs is a new way to unveil the molecular mechanisms underlying OLP and might represent a novel candidate biomarker for diagnosis [8, 9] which supports the current data. Although it is not possible to identify the exact role of miR-138 in OLP, some hypothesis could be drawn. Recent reports provided explanation

**Fig. 3** miR-138 relative gene expression levels were inversely correlated with CCND1 index (protein expression) in OLP

![Graph showing the inverse correlation between miR-138 and CCND1 expression](image)

\[ R^2 \text{ Linear} = 0.165 \]
for how miR-138 could regulate the immune response in some chronic diseases through the action of other regulatory mechanisms [59, 60]. Fu et al. [59] showed that miR-138 expression was decreased significantly in psoriasis patients compared with healthy controls, suggesting that the downregulation of miR-138 induced an imbalance in Th1/Th2, involved in the pathogenesis of psoriasis. It is well established that OLP is characterized by the imbalance between the levels of Th1 and Th2 immune responses [3]; consequently, this study hypothesized that miR-138 downregulation might contribute to the intense inflammation seen in OLP through its impact on Th1 and Th2. This might be one of the mechanisms that may be implicated in OLP pathogenesis. Furthermore, subgroup analysis revealed that miR-138 expression was significantly lower in cases of atrophic and erosive OLP than reticular OLP group. Similarly, Zhang et al. [54] revealed that miR-27b was significantly lower in atrophic and erosive OLP compared with reticular OLP suggesting that downregulation of miR-27b was associated with the disease activity. Hu et al. [57] also showed that miR-125a has varied expression in different clinical forms of OLP and might be a novel candidate biomarker to estimate the severity of OLP. Along with the current

Fig. 4 miR-138 relative gene expression levels were inversely correlated with CCND1 relative gene expression in OLP

Fig. 5 CCND1 relative gene expression levels were positively correlated with CCND1 protein expression in OLP
litterature which confirms that erosive and atrophic OLP show more severe clinical symptoms, pathological features, and differ in treatment than reticular OLP [61, 62], this study supports that different clinical forms of OLP may have a distinct immune modulatory background.

Despite current established evidence in the literature considering erosive and atrophic subtypes of OLP to have a higher risk of malignant transformations, still the mechanism is poorly understood [2, 6]. Carcinogenesis can occur as a result of uncontrolled cell proliferation due to multiple genetic alterations associated with aberrant cell cycle regulation [35]. Consistent with this hypothesis, previous studies showed that overexpression of CCND1 was implicated in the pathogenesis of HNSCC [35, 63, 64]. Furthermore, Bascones et al. [65] suggested that molecular alterations related to cell cycle control may produce an epithelial substrate that favors evolution to malignity in OLP. Since erosive and atrophic OLP epithelium is more susceptible to malignant transformation being more sensitive to carcinogenic exposures than normal oral mucosa [5, 52], action on proliferating cells by oncogenic insults may lead to the development of a malignant cell phenotype in OLP patients [65]. Accordingly, this study agrees with other reports suggesting that overexpression of CCND1 at both protein and gene levels in OLP may disturb the normal cell cycle control providing tumor cells with a selective growth advantage [36, 38]. Moreover, Rezaee et al. [52] reported that the increased cell proliferation observed in OLP favors the accumulation of cell cycle genetic alterations which might be considered as a strong indicator of malignant potential in OLP, which agrees with the current results.

Previous reports suggested that miR-138 might function as a tumor suppressor by negatively regulating cyclin genes in cancer [66]. Earlier studies showed that CCND1 and D3 levels were inversely correlated with miR-138 expression in nasopharyngeal carcinoma [33] and hepatocellular carcinoma [27]. Numerous investigations also observed that dysregulation of miR-138 might contribute to the enhanced cell migration in HNSCC [26, 29–31]. Interestingly, this study demonstrated an inverse correlation between miR-138 and CCND1 expression in OLP patients. This is in agreement with Liu et al. [33], this study suggested that miR-138 negatively regulates cell cycle progression in OLP by targeting critical cell cycle regulators leading to overexpression of CCND1 and eventually cell proliferation. Recently, Yang et al. [62] reported that miR-146a may be involved in the malignancy associated with erosive OLP and could be used as a potential biological marker to evaluate the severity of OLP. Based on the abovementioned data, it might be speculated that miRNA-138 might be considered as a tumor suppressor in premalignant lesions like atrophic and erosive OLP. However, further studies involving HNSCC are warranted to confirm this speculation.

In conclusion, this investigation showed that the downregulation of miR-138 increases the expression of their potential targets CCND1 in OLP mucosa which might indicate a pivotal role in the disease pathogenesis. The present data highlights the potential role of miRNA expression analysis in OLP, which will ultimately improve diagnosis and patient outcome. Moreover, this study suggests that miR-138 might be considered as a potential novel therapeutic target for atrophic and erosive OLP patients. Further characterization of miRNA and their target genes will advance our understanding of the pathogenesis of OLP, and a large-scale patient-based study will be needed to fully explore the mechanism that regulates the expression of this noncoding gene.

Compliance with ethical standards

Conflict of interest Dr. Noha Ghallab declares that she has no conflict of interest. Dr. Rehab Kasem declares that she has no conflict of interest. Dr. Safa Abd El-Ghani declares that she has no conflict of interest. Dr. Olfat Shaker declares that she has no conflict of interest.

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Ethical approval This article was approved by the Faculty of Oral and Dental Medicine Research Ethics Committee, Cairo University in October 2014.

Informed consent Following an explanation of the study as well as information about the sampling procedures, each subject signed a written informed consent form approved by the Faculty Research Ethics Committee.

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