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Long non-coding RNA DQ786243 modulates the induction and function of $CD4^+$ Treg cells through Foxp3-miR-146a-NF-κB axis: Implications for alleviating oral lichen planus

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ABSTRACT

The present study was aimed to investigate the effects of the long non-coding RNA DQ786243 in the regulation of Treg cells in oral lichen planus (OLP), as well as to evaluate its potential molecular mechanisms. Here we found that the expression of DQ786243 and Foxp3 were both overexpressed in the $CD4^+$ cells from the peripheral blood of OLP patients, and their expression was positively correlated. Meanwhile, compared with the normal $CD4^+$ cells, the frequency of $Foxp3^+$ Treg cells in the OLP $CD4^+$ cells was significantly higher. DQ786243 overexpression in normal $CD4^+$ cells resulted in the upregulation of Foxp3 and the higher frequency of $Foxp3^+$ Treg cells. Furthermore, we found that the induction of $Foxp3^+$ Treg cells by DQ786243 significantly increased its suppressive function, and suppressed the function of other $CD4^+$ T cells such as Th1 and Th17 by decreasing the levels of IFN-γ and IL-17. Moreover, we found that DQ786243 overexpression markedly elevated the expression of miR-146a via regulating Foxp3, and thus inhibiting the NF-κB signaling. In conclusion, these findings indicate that DQ786243 may regulate the induction and function of $CD4^+$ Treg cells through Foxp3-miR-146a-NF-κB axis, implicating a novel insight into understanding the progression of OLP.

1. Introduction

Oral lichen planus (OLP) is a common chronic mucocutaneous disease that has a higher morbidity in women than men, which affects about 0.5% to 2.2% of the general adult population [1,2]. This disease is characterized histologically by the keratinization of epithelial cells, the lymphocyte infiltration into the subepithelial region, and the degeneration of the basal layer [3,4]. Once established, typical oral lesions rarely undergo self-remission. It has been reported that erythematous and erosive lesions are often sensitive or painful, which seriously affect the quality of life of patients. Moreover, more and more studies showed that OLP may be premalignant [5,6].

Emerging evidence shows that the dysregulation of immune system plays a crucial role in the pathogenesis of OLP [7]. As a subset of T lymphocytes, $CD4^+CD25^+$ regulatory T (Treg) cells have been shown to be deeply involved in the induction and maintenance of immunologic tolerance to self and non-self [8,9]. Forkhead box P3 (Foxp3) is a transcription factor that is specifically expressed in naturally $CD4^+$ Treg cells. It has been reported that in an autoimmune and inflammatory syndrome in humans and mice, Foxp3 is genetically defective [10,11]. Foxp3 has been identified as an important regulatory gene for the development of Treg cells [12–14]. Recently, abnormal number and function of Foxp3-expressing $CD4^+CD25^+$ Treg cells have been found in various autoimmune and inflammatory diseases, such as psoriasis [15], multiple sclerosis [16], rheumatoid arthritis [17], and OLP [18]. Moreover, it has been indicated that inducing Foxp3-expressing $CD4^+CD25^+$ Treg cells may be a potential therapy for autoimmune and inflammatory diseases.

Long noncoding RNAs (lncRNAs) are noncoding transcripts longer than 200 nucleotides, which has been identified as new regulatory molecules [19]. It has been shown that lncRNAs modulate protein-coding gene at the chromatin remodeling level, or the transcriptional and post-transcriptional control level [20,21]. They exert crucial roles by acting as decoys, guides, signals, and scaffolds [22]. Although their characteristics are much less known compared with microRNAs (miRNAs) [23], emerging studies show that lncRNAs play vital roles in cell biology, such as stem cell development, cell differentiation, cell growth and apoptosis, etc. [24–26]. DQ786243, a newly identified

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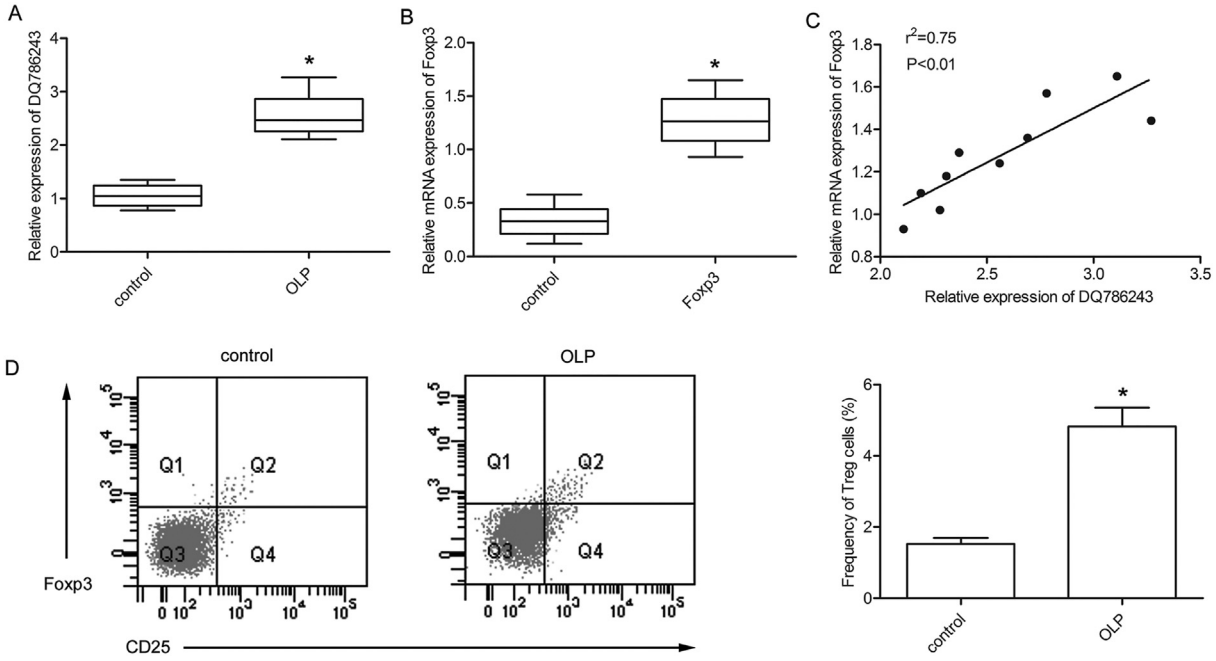

Fig. 1. The expression of DQ786243 and Foxp3 was up-regulated in $CD4^+$ cells from OLP patients. Control group, $CD4^+$ cells from normal samples; OLP group, $CD4^+$ cells from OLP patients. (A) The expression level of DQ786243 (A) and Foxp3 (B) were measured by qRT-PCR assay. (C) Correlation between the expression of DQ786243 and Foxp3 in the $CD4^+$ cells from OLP patients. (D) The frequency of Treg cells was detected by flow cytometry. *$P < .05$ versus the control group.

lncRNA, was firstly reported to be overexpressed in hepatocellular carcinoma (HCC) [27]. Thereafter, aberrant expression of DQ786243 was found in several types of cancers, such as colorectal cancer [28], gastric cancer [29,30] and HCC [31]. However, the expression and roles of DQ786243 in many inflammatory disorders including OLP was still unknown. Qiao et al. have reported that DQ786243 affects Foxp3 expression in Treg cells of Crohn's disease [32]. Thus, we speculated whether DQ786243 played a crucial role in OLP via modulating $Foxp3^+$ Treg cells.

In the present study, we aimed to investigate the expression and effects of DQ786243 in the modulation of $Foxp3^+$ Treg cells in OLP, and to evaluate the underlying molecular mechanism, in order to better understand the pathogenesis of OLP and provide effective targets for OLP therapy.

2. Materials and methods

2.1. Human subjects

Blood samples from 10 healthy donors and 10 OLP patients were recruited after written informed consent was obtained from the First Affiliated Hospital of Zhengzhou University. The research protocols were approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University.

2.2. $CD4^+$ cell isolation

PBMCs were isolated from blood samples by Ficoll density gradient centrifugation. The cells were immediately stained with monoclonal antibody CD4 and its appropriate isotype control according to the recommendations of the manufacturer (eBioscience, San Diego, CA, USA) for flow cytometry. Then at least 2×10^4 $CD4^+$ lymphocytes were acquired for the following analysis of Treg cells [4].

2.3. Cell transfection

pLentiLox3.7-DQ786243 plasmids were constructed by Sangon Biotech (Shanghai, China), and the empty pLentiLox3.7 plasmids were used as corresponding negative control. Small interference RNA (siRNA) against Foxp3 (si-Foxp3), and corresponding negative control (si-NC), miR-146a mimic and mimic negative control (mimic-NC) were obtained from GenePharma Co., Ltd. (Shanghai, China). The cell transfection was performed by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.4. qRT-PCR [33]

RNA was isolated by Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed to cDNA using SuperScript First Strand cDNA System (Invitrogen). The mRNA levels of DQ786243, Foxp3, GranB, CTLA-4, GITR, TGF-β, IL-10, IFN-γ, and IL-17 were measured by the SYBR Premix Ex Taq kit (Takara, Dalian, China). The levels of miR-146a were measured by TaqMan microRNA assays (Applied Biosystems, Foster City, CA, USA). The relative expression of miR-146a was normalized to U6, and the relative expression of DQ786243 and gene mRNA was normalized to GAPDH. The data were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.5. Treg cells analysis

The percentage of Treg cells in $CD4^+$ T cells was analyzed by flow cytometry. Briefly, the $CD4^+$ T cells were stained with FITC-labeled anti-CD25 and PE-labeled anti-Foxp3 (BD PharMingen, San Diego, CA), respectively, for 30 min at room temperature. The data were analyzed by CELL Quest software [34].

2.6. Western blot

Protein was extracted by RIPA protein extraction reagent (Beyotime). Equal amounts (50 μg) of protein were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Millipore, Billerica, MA, USA). After blocking in 5% non-fat milk for 1 h at room temperature, the membranes were incubated with primary antibodies (anti-Foxp3, anti-p-IκB-α, anti-p-p65) at 4 °C overnight. After washing 3

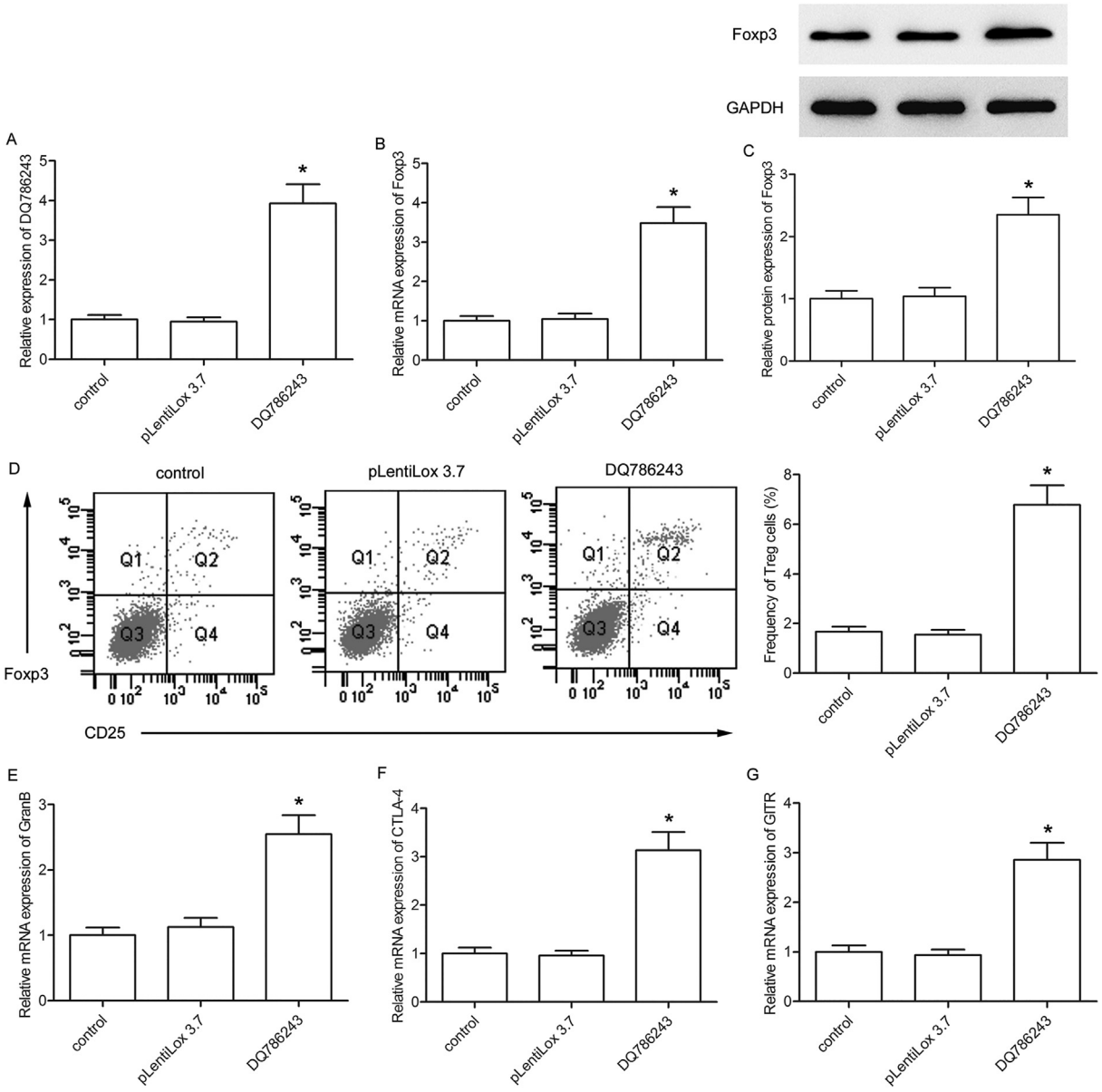

Fig. 2. DQ786243 overexpression induced Foxp3 expression and Treg cells. Control group, $CD4^+$ cells from normal samples; pLentiLox 3.7 group, $CD4^+$ cells transfected with pLentiLox 3.7 empty plasmids; DQ786243 group, $CD4^+$ cells transfected with pLentiLox 3.7-DQ786243 plasmids. (A) The expression level of DQ786243 was measured by qRT-PCR assay. (B) The mRNA expression level of Foxp3 was measured by qRT-PCR assay. (C) The protein expression level of Foxp3 was measured by western blot assay. (D) The frequency of Treg cells was detected by flow cytometry. The mRNA expression levels of GranB (E), CTLA-4 (F) and GITR (G) were measured by qRT-PCR assay. $^*P < .05$ versus the control group or the pLentiLox 3.7 group.

times, the membranes were incubated with horseradish-peroxidase-conjugated secondary antibodies for 1 h at room temperature. The antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA) and Abcam (Cambridge, MA, USA). They were all used at the manufacturers' recommended dilutions. The bands were visualized by an enhanced chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ, USA). The relative protein expression was normalized to GAPDH expression.

2.7. In vitro proliferation assay

The in vitro proliferation assay to analyze the direct suppressive capacity of $CD4^+CD25^+Foxp3^+$ Treg cells to $CD4^+CD25^-$ T cells was performed as previously described [4]. $CD4^+CD25^+Foxp3^+$ Treg cells form different treated groups, and $CD4^+CD25^-$ T cells form the healthy donors (normal controls) were isolated by cell sorting. The $CD4^+CD25^-$ T cells were labeled with carboxyfluorescein succinimidyl ester (CFSE, Invitrogen), and stimulated with anti-CD3 mAb (BD Pharmingen) and anti-CD28 mAb (BD Pharmingen). Then $CD4^+CD25^-$ T cells (4×10^4 cells/well) were co-cultured without or with different numbers of Treg cells ($CD4^+CD25^-$ T cells:Treg cells = 1: 0, 1: 0.5, and 1: 1) in 96-well plates. After 72 h, the cell proliferation by CFSE dilution was determined by flow cytometry. The proliferation inhibition rate = (the percentage of dividing CFSE-labeled $CD4^+CD25^-$ T cells cultured alone - the percentage of dividing CFSE-labeled $CD4^+CD25^-$ T cells in the co-culture)/the percentage of dividing CFSE-labeled $CD4^+CD25^-$ T cells cultured alone × 100%.

2.8. Statistical analysis

All data were analyzed with GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). They are shown as the mean ±

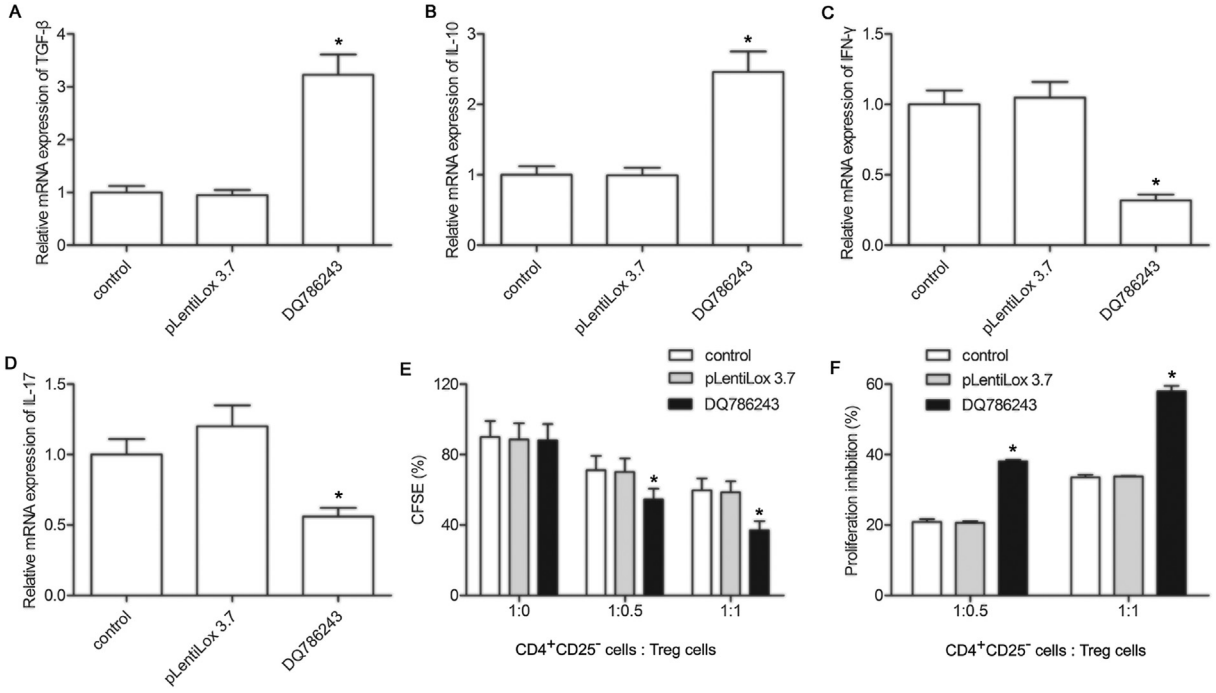

Fig. 3. DQ786243 overexpression suppressed the expression of IFN-γ and IL-17 and promoted the suppressive activity of Treg cells. Control group, $CD4^+$ cells from normal samples; pLentiLox 3.7 group, $CD4^+$ cells transfected with pLentiLox 3.7 empty plasmids; DQ786243 group, $CD4^+$ cells transfected with pLentiLox 3.7-DQ786243 plasmids. The mRNA expression levels of TGF-β (A) and IL-10 (B), IFN-γ (C) and IL-17 (D) were measured by qRT-PCR assay. (E) The $CD4^+CD25^-$ T cells labeled with CFSE were co-cultured without or with Treg cells ($CD4^+CD25^-$ T cells:Treg cells = 1: 0, 1: 0.5, and 1: 1) isolated from different groups. After 72 h, the cell proliferation by CFSE dilution was determined by flow cytometry. (F) The proliferation inhibition rate was calculated by (the percentage of dividing CFSE-labeled $CD4^+CD25^-$ T cells cultured alone - the percentage of dividing CFSE-labeled $CD4^+CD25^-$ T cells in the co-culture)/the percentage of dividing CFSE-labeled $CD4^+CD25^-$ T cells cultured alone × 100%. *$P < .05$ versus the control group or the pLentiLox 3.7 group.

standard deviation (SD) from a minimum of 3 replicates. *t*-Test was used to compare 2 groups, and a one-way analysis of variance was used to compare more than 2 groups. Differences at $P < .05$ were considered statistically significant.

3. Results

3.1. The expression of DQ786243 and Foxp3 was up-regulated in $CD4^+$ cells from OLP patients

To investigate whether the DQ786243 and Foxp3 was involved in the pathology of OLP, we measured their expression in the $CD4^+$ cells from the PMBCs of OLP patients and normal controls. As shown in Fig. 1A, compared with the control group, the expression of DQ786243 in the OLP group was significantly up-regulated. The same result was seen in the expression of Foxp3, and the Foxp3 in the OLP group was markedly up-regulated as compared with the control group (Fig. 1B). Furthermore, we found that the expression of DQ786243 and Foxp3 in the OLP group showed a positive correlation (Fig. 1C). As shown in Fig. 1D, a flow cytometry assay indicated that the frequency of $Foxp3^+$ Treg cells in the OLP group was dramatically increased as compared with that in the control group.

3.2. DQ786243 overexpression induced Foxp3 expression and Treg cells

To investigate the roles of DQ786243 in the Treg cells, we overexpressed it in the $CD4^+$ cells from the normal controls (Fig. 2A). Then we found that the mRNA and protein expression of Foxp3 in the DQ786243 group was both increased as compared with that in the pLentiLox 3.7 group (Fig. 2, B and C). The frequency of $Foxp3^+$ Treg cells in the DQ786243 group was also higher than that in the pLentiLox 3.7 group (Fig. 2D). Moreover, we detected the mRNA expression levels of three Treg-related genes, as shown in Fig. 2, E–G, compared with the pLentiLox 3.7 group, the mRNA expression of GranB, CTLA-4 and GITR in the DQ786243 group showed obvious up-regulation.

3.3. DQ786243 overexpression suppressed the expression of IFN-γ and IL-17 and promoted the suppressive activity of Treg cells

Given the previous data that DQ786243 overexpression induced increased $Foxp3^+$ Treg cells, we further evaluated the suppressive activity of the Treg cells. We measured the mRNA expression of two Treg-related cytokines, and found that compared with the pLentiLox 3.7 group, the mRNA expression of TGF-β and IL-10 in the DQ786243 group was significantly elevated (Fig. 3, A and B). Furthermore, we found that compared with the pLentiLox 3.7 group, the mRNA expression of IFN-γ and IL-17 in the DQ786243 group was significantly suppressed (Fig. 3, C and D). In addition, we performed the in vitro proliferation assay to analyze the direct suppressive activity of $Foxp3^+$ Treg cells to $CD4^+CD25^-$ T cells. As shown in Fig. 3, E and F, compared with the pLentiLox 3.7 group, Treg cells form DQ786243 group significantly reduced the proliferation of $CD4^+CD25^-$ T cells at either 1:0.5 or 1:1 ratio ($CD4^+CD25^-$ T cells:Treg cells).

3.4. DQ786243 overexpression effected Foxp3-miR-146a-NF-κB axis

To investigate whether the effects of DQ786243 overexpression was associated with Foxp3 overexpression, we transfected pLentiLox 3.7-DQ786243 along with Foxp3 siRNAs into the $CD4^+$ cells from the normal samples. As shown in Fig. 4, A and B, compared with the DQ786243 + si-NC group, the mRNA and protein expression of Foxp3 in the DQ786243 + si-Foxp3 group was significantly down-regulated. We further found that DQ786243 overexpression induced the increase of miR-146a expression, which was suppressed by the co-transfection of LentiLox 3.7-DQ786243 and Foxp3 siRNAs (Fig. 4C). In consideration of the effects of miR-146a on NF-κB signaling, we further evaluated the

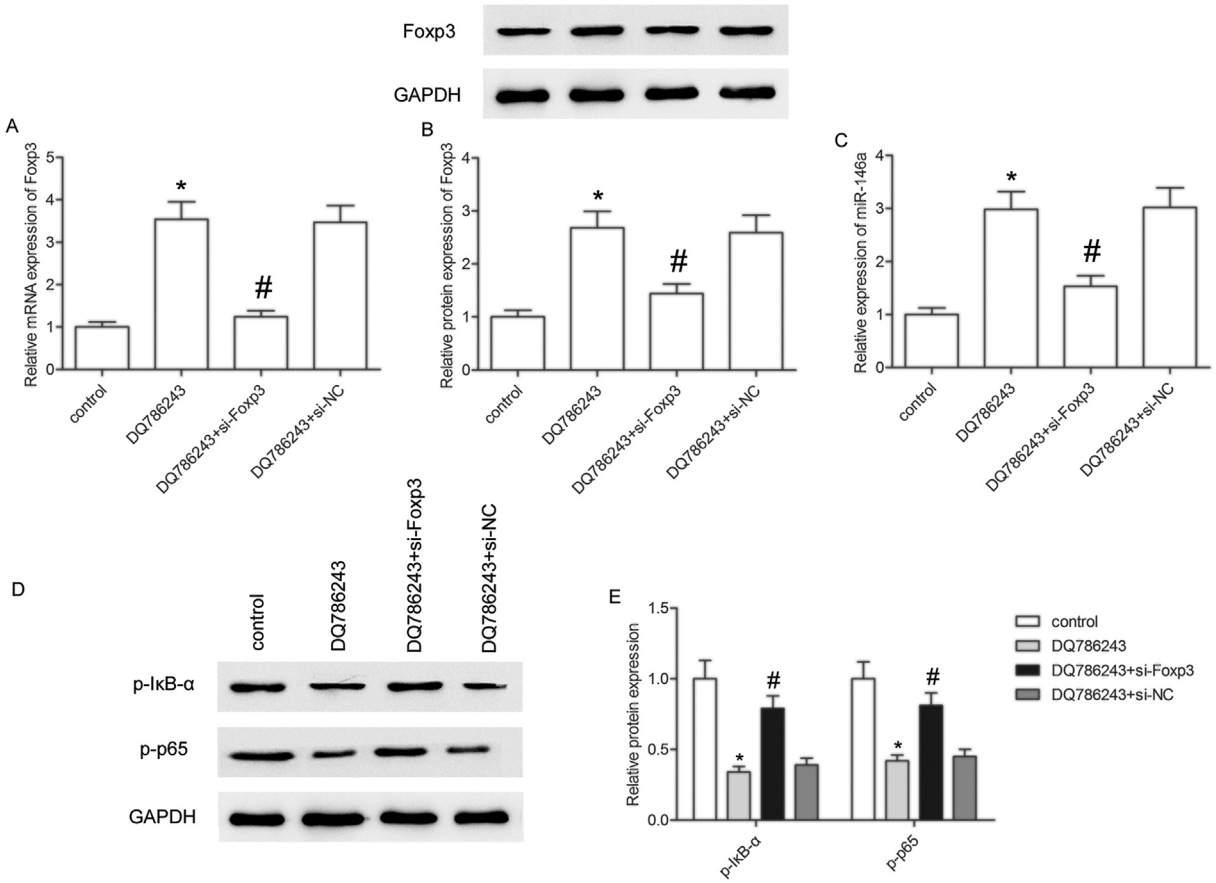

Fig. 4. DQ786243 overexpression effected Foxp3-miR-146a-NF-κB axis. Control group, $CD4^+$ cells from normal samples; DQ786243 group, $CD4^+$ cells transfected with pLentiLox 3.7-DQ786243 plasmids; DQ786243 + si-Foxp3 group, $CD4^+$ cells co-transfected with pLentiLox 3.7-DQ786243 plasmids and Foxp3 siRNAs; DQ786243 + si-NC group, $CD4^+$ cells co-transfected with pLentiLox 3.7-DQ786243 plasmids and siRNA negative controls. The mRNA (A) and protein (B) expression levels of Foxp3 were measured by qRT-PCR and western blot assay, respectively. (C) The expression level of miR-146a was measured by qRT-PCR assay. (D, E) The protein expression levels of p-IκB-α and p-p65 were measured by western blot assay. $*P < .05$ versus the control group; $\#P < .05$ versus the DQ786243 group.

expression of p-IκB-α and p-p65. As shown in Fig. 4, D and E, compared with the control group, the expression of p-IκB-α and p-p65 was significantly decreased in the DQ786243 group; compared with the DQ786243 group, the expression of p-IκB-α and p-p65 was significantly elevated in the DQ786243 + si-Foxp3 group. The above date suggest that when the DQ786243 was overexpressed, the Foxp3-miR-146a-NF-κB axis changed.

3.5. Foxp3-miR-146a-NF-κB axis partly mediated the effects of DQ786243 overexpression

To investigate whether the effects of DQ786243 overexpression was associated with Foxp3-miR-146a-NF-κB axis, we transfected pLentiLox 3.7-DQ786243 along with Foxp3 siRNAs into the $CD4^+$ cells from the normal samples, followed by the transfection of miR-146a mimic or the treatment of PDTC. Then we measured the mRNA expression of TGF-β, IL-10, IFN-γ and IL-17. As shown in Fig. 5, A and B, compared with the DQ786243 group, the elevated mRNA expression of TGF-β and IL-10 was significantly decreased in the DQ786243 + si-Foxp3 group; compared with the DQ786243 + si-Foxp3 group, the expression of TGF-β and IL-10 showed an obvious elevation in the DQ786243 + si-Foxp3 + miR-146a group or the DQ786243 + si-Foxp3 + PDTC group. As shown in Fig. 5, C and D, the DQ786243 overexpression-induced decreased expression of IFN-γ and IL-17 showed an increase in the DQ786243 + si-Foxp3 group; and further showed a decrease in the DQ786243 + si-Foxp3 + miR-146a group or the DQ786243 + si-Foxp3 + PDTC group as compared with the DQ786243 + si-Foxp3 group. The above data suggest that the effects of DQ786243 overexpression on Treg cells were partly mediated by the Foxp3-miR-146a-NF-κB axis.

4. Discussion

In recent years, lncRNAs have been attracting more attention in the pathogenesis of a variety of diseases. As a newly identified lncRNA, the roles of DQ786243 have been confirmed in several cancers and Crohn's disease. In the present study, we tried to investigate the effects of DQ786243 in the progression of OLP. It has been reported that DQ786243 affects Treg related Foxp3 expression [32], and $Foxp3^+$ Treg cells plays an important role in the development of OLP, we hypothesized that the dysregulation of DQ786243 was deeply involved in the OLP via modulating $Foxp3^+$ Treg cells.

In the present study, we first evaluated the expression of DQ786243 and Foxp3 in the $CD4^+$ cells from the PMBCs of OLP patients and normal controls, and found that their expression both showed up-regulation in the OLP group, suggesting that the dysregulation of DQ786243 and Foxp3 contributed to the development of OLP. A positive correlation between the DQ786243 and Foxp3 in the OLP group was found, indicating that DQ786243 affected Foxp3 expression, which was consistent with the previous study [32]. Meanwhile, we found that the frequency of $Foxp3^+$ Treg cells in the OLP group was dramatically higher than that in the control group, suggesting that there may be a close relation between DQ786243 and $Foxp3^+$ Treg cells. To investigate the roles of DQ786243 in the Treg cells, we overexpressed it in the $CD4^+$ cells from the normal samples. Then we found that DQ786243 overexpression significantly increased the Foxp3 expression,

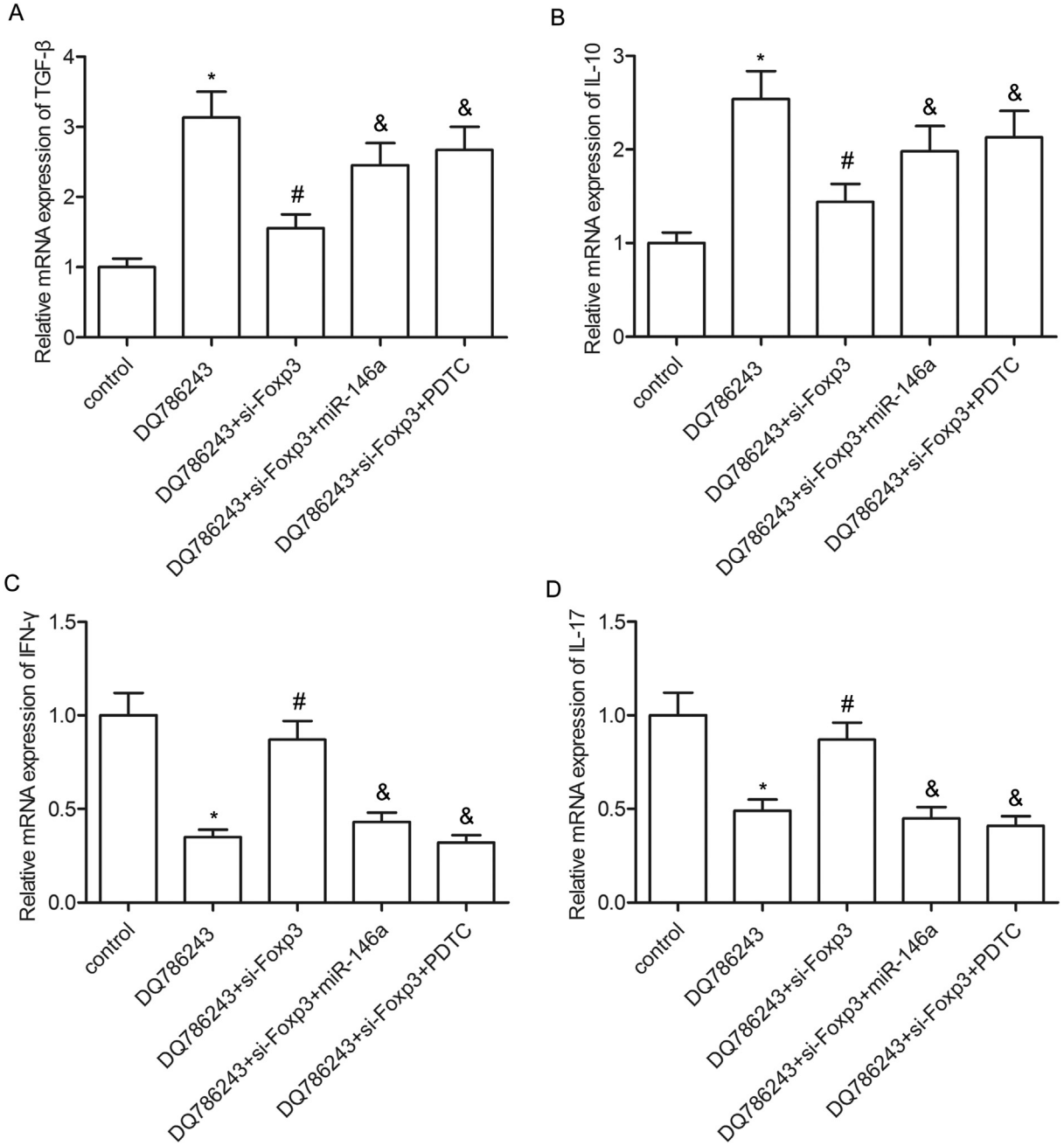

Fig. 5. Foxp3-miR-146a-NF-κB axis partly mediated the effects of DQ786243 overexpression. Control group, $CD4^+$ cells from normal samples; DQ786243 group, $CD4^+$ cells transfected with pLentiLox 3.7-DQ786243 plasmids; DQ786243 + si-Foxp3 group, $CD4^+$ cells co-transfected with pLentiLox 3.7-DQ786243 plasmids and Foxp3 siRNAs; DQ786243 + si-Foxp3 + miR-146a group, $CD4^+$ cells co-transfected with pLentiLox 3.7-DQ786243 plasmids, Foxp3 siRNAs and miR-146a mimics; DQ786243 + si-Foxp3 + PDTC group, $CD4^+$ cells co-transfected with pLentiLox 3.7-DQ786243 plasmids and Foxp3 siRNAs, followed by the treatment of PDTC. The mRNA expression levels of TGF-β (A) and IL-10 (B), IFN-γ (C) and IL-17 (D) were measured by qRT-PCR assay. *$P < .05$ versus the control group; #$P < .05$ versus the DQ786243 group; &$P < .05$ versus the DQ786243 + si-Foxp3 group.

elevated the frequency of $Foxp3^+$ Treg cells, and up-regulated the expression of Treg-related genes (GranB, CTLA-4 and GITR). The above data indicated that DQ786243 dysregulation was involved in the progression of OLP via modulating $Foxp3^+$ Treg cells.

It has been reported that in addition to Treg frequency, the functional activity of Treg cells was also crucial to autoimmune and inflammatory diseases [35]. The activity of Treg cells to suppress T cell immune responses in patients with multiple sclerosis is decreased while the number of the cells is unaltered [36,37]. Similarly, the suppressive activity of Treg cells isolated from active rheumatoid arthritis patients was markedly decreased [38]. And Zhou et al. have reported that in patients with OLP, the Treg cells were frequently increased but functionally impaired [4]. These results suggest that to increase the functional Treg cells may be therapeutic. To further evaluate the functional activity of DQ786243 overexpression-induced $Foxp3^+$ Treg cells, we firstly measured the expression of two Treg-related cytokines (TGF-β and IL-10), and found that their expression was significantly elevated by the DQ786243 overexpression. Moreover, $Foxp3^+$ Treg cells maintain immune homeostasis by suppressing different inflammatory responses [39]. The Th1 cell marker IFN-γ and Th17 cell marker IL-17 [40] in the DQ786243 group was significantly suppressed. Furthermore, the in vitro proliferation assay showed that DQ786243

overexpression-induced Foxp3$^+$ Treg cells possessed higher suppressive capacity to inhibit the proliferation of CD4$^+$CD25$^-$ T cells. The above data suggested that DQ786243 overexpression induced the functional Foxp3$^+$ Treg cells.

To further confirm whether the effects of DQ786243 overexpression was associated with Foxp3 overexpression, we transfected pLentiLox 3.7-DQ786243 along with Foxp3 siRNAs into the CD4$^+$ cells from the normal samples to suppress DQ786243 overexpression-induced Foxp3 expression. Foxp3 was reported to regulate miR-146a expression, and miR-146a is critical for the suppressive activity of Foxp3$^+$ Treg cells [39]. And one our previous study also showed that Foxp3 controls progression of OLP by regulating miR-146a, further negatively regulating TRAF6 [41]. We further found that DQ786243 overexpression induced the increase of miR-146a expression, which was suppressed by the knockdown of Foxp3. It is well established that miR-146a negatively regulates NF-kB activation by suppressing IRAK1 and TRAF6 expression [42,43]. Therefore, we further evaluated the expression of p-IκB-α and p-p65 to assess the NF-κB activation. The results showed that the DQ786243 overexpression significantly decreased the expression of p-IκB-α and p-p65, which showed an elevation in the DQ786243 + si-Foxp3 group. The above date showed that when the DQ786243 was overexpressed, the Foxp3-miR-146a-NF-κB axis changed, suggesting that the Foxp3-miR-146a-NF-κB axis may be related to the effects of DQ786243.

Zhang et al. have reported that NF-κB activation negatively correlates with Treg Cell frequency in infiltrated mononuclear cells in OLP patients [44], suggesting that there are close relation between NF-κB signaling and Treg cells. To investigate whether the effects of DQ786243 overexpression was associated with Foxp3-miR-146a-NF-κB axis, we transfected pLentiLox 3.7-DQ786243 along with Foxp3 siRNAs into the CD4$^+$ cells from the normal samples, followed by the transfection of miR-146a mimic or the treatment of PDTC. We found that the DQ786243 overexpression-induced elevated expression of TGF-β and IL-10 was significantly decreased in the DQ786243 + si-Foxp3 group, but showed an obvious elevation in the DQ786243 + si-Foxp3 + miR-146a group or the DQ786243 + si-Foxp3 + PDTC group. And the DQ786243 overexpression-induced decreased expression of IFN-γ and IL-17 showed an increase in the DQ786243 + si-Foxp3 group, but further showed a decrease in the DQ786243 + si-Foxp3 + miR-146a group or the DQ786243 + si-Foxp3 + PDTC group. The above data suggest that the effects of DQ786243 overexpression on the function of Treg cells were partly mediated by the Foxp3-miR-146a-NF-κB axis.

In summary, these results demonstrate that the dysregulation of DQ786243 was deeply involved in the modulation of Treg cells in OLP via Foxp3-miR-146a-NF-κB axis, implicating a novel insight into understanding the progression of OLP. Overexpression of DQ786243 induced functional Foxp3$^+$ Treg cells, revealing a potential therapeutic strategy for OLP, even the autoimmune and inflammatory diseases.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

[1] A. Mansourian, N. Shanbehzadeh, S.J. Kia, M.S. Moosavi, Increased salivary aldehyde dehydrogenase 1 in non-reticular oral lichen planus, An. Bras. Dermatol. 92 (2017) 168–171.
[2] N.G. Bediaga, X. Marichalar-Mendia, J.M. Aguirre-Urizar, et al., Global DNA methylation: uncommon event in oral lichenoid disease, Oral Dis. 20 (2015) 821–826.
[3] F.A.C.G.D. Sousa, T.C. Paradella, L.E.B. Rosa, Oral lichen planus versus epithelial dysplasia: difficulties in diagnosis, Brazilian Journal of Otorhinolaryngology 75 (2009) 716–720.
[4] L. Zhou, T. Cao, Y. Wang, et al., Frequently increased but functionally impaired CD4+CD25+ regulatory T cells in patients with oral lichen planus, Inflammation 39 (2016) 1205–1215.
[5] B. Lončar-Brzak, M. Klobučar, I. Veliki-Dalić, et al., Expression of small leucine-rich extracellular matrix proteoglycans biglycan and lumican reveals oral lichen planus malignant potential, Clin. Oral Investig. 22 (2018) 1071–1082.
[6] E.A. Georgakopoulou, M.D. Achtari, M. Achtaris, et al., Oral lichen planus as a preneoplastic inflammatory model, J. Biomed. Biotechnol. 2012 (2012) 759626.
[7] X.A. Tao, J. Xia, X.B. Chen, et al., FOXP3 T regulatory cells in lesions of oral lichen planus correlated with disease activity, Oral Dis. 16 (2010) 76–82.
[8] S. Sakaguchi, Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self, Nat. Immunol. 6 (2005) 345–352.
[9] X. He, H.J. Koenen, J.H. Slaats, I. Joosten, Stabilizing human regulatory T cells for tolerance inducing immunotherapy, Immunotherapy 9 (2017) (imt-2017-0017).
[10] L. Lu, J. Barbi, F. Pan, The regulation of immune tolerance by FOXP3, Nat. Rev. Immunol. 17 (2017) 703.
[11] B. Min, Heterogeneity and stability in Foxp3+ regulatory T cells, J. Interf. Cytokine Res. 37 (2017) 386–397.
[12] S. Hori, T. Nomura, S. Sakaguchi, Control of regulatory T cell development by the transcription factor Foxp3, Science 299 (2003) 1057–1061.
[13] Z. Lianjun, Z. Yong, The regulation of Foxp3 expression in regulatory CD4(+)CD25(+)T cells: multiple pathways on the road, J. Cell. Physiol. 211 (2010) 590–597.
[14] S.A. Oh, M. Liu, B.G. Nixon, et al., Foxp3-independent mechanism by which TGF-β controls peripheral T cell tolerance, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E7536.
[15] K. Yan, W. Xu, Y. Huang, et al., Methotrexate restores the function of peripheral blood Treg cells in psoriasis vulgaris via CD73/AMPK/mTOR pathway, Br. J. Dermatol. 179 (2018) 896–905.
[16] K. Kimura, H. Hohjoh, M. Fukuoka, et al., Circulating exosomes suppress the induction of regulatory T cells vialet-7iin multiple sclerosis, Nat. Commun. 9 (2018) 17.
[17] P. Zafari, K. Yari, S. Mostafaei, et al., Analysis of Helios gene expression and Foxp3 TSDR methylation in the newly diagnosed Rheumatoid Arthritis patients, Immunol. Investig. 10 (2018) 1–11.
[18] T. X-A, J. Xia, C. X-B, et al., FOXP3 T regulatory cells in lesions of oral lichen planus correlated with disease activity, Oral Dis. 16 (2010) 76–82.
[19] C.P. Ponting, P.L. Oliver, W. Reik, Evolution and functions of long noncoding RNAs, Cell 136 (2009) 629–641.
[20] A.M. Khalil, G. Mitchell, H. Maite, et al., Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression, Proceedings of the National Academy of Sciences of the United States of America, 2009.
[21] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (2009) 155–159.
[22] K. Wang, H. Chang, Molecular mechanisms of long noncoding RNAs, Mol. Cell 43 (2011) 904–914.
[23] H. H-W, J.T. Mendell, MicroRNAs in cell proliferation, cell death, and tumorigenesis, Br. J. Cancer 94 (2006) 776–780.
[24] G. Mitchell, D. Julie, B.W. Carey, et al., lincRNAs act in the circuitry controlling pluripotency and differentiation, Nature 477 (2011) 295.
[25] L. Sabine, M.N. Cabili, G. Mitchell, et al., Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells, Nat. Genet. 42 (2010) 1113–1117.
[26] K.C. Wang, Y.W. Yang, L. Bo, et al., A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression, Nature 472 (2011) 120–124.
[27] Y. Fu, Z. Ling, H. Xi-Song, et al., Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans, Hepatology 54 (2011) 1679–1689.
[28] L. Sun, H. Xue, C. Jiang, et al., LncRNA DQ786243 contributes to proliferation and metastasis of colorectal cancer bothin vitroandin vivo, Biosci. Rep. 36 (2016) e00328.
[29] M.H. Zhang, Y. Yang, Y. Zhao, et al., LncRNA DQ786243 expression as a biomarker for assessing prognosis in patients with gastric cancer, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 2304–2309.
[30] T. Shan, J. Fan, Q. Zhao, et al., Upregulation of long non-coding RNA DQ786243 promotes the progression of gastric cancer, Mol. Med. Rep. 16 (2017) 3761–3768.
[31] B. Zeng, Z. Lin, H. Ye, et al., Upregulation of LncDQ is associated with poor prognosis and promotes tumor progression via epigenetic regulation of the EMT pathway in HCC, Cellular Physiology & Biochemistry 46 (2018) 1122–1133.
[32] Q.Y. Qi, H.M. Lan, X.A. Tao, et al., LncRNA DQ786243 affects Treg related CREB and Foxp3 expression in Crohn's disease, J. Biomed. Sci. 20 (2013) 87.
[33] X. Han, Q. Wang, Y. Wang, et al., Long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1/microRNA-202-3p/periostin axis modulates invasion and epithelial-mesenchymal transition in human cervical cancer, J. Cell. Physiol. 234 (2019) 14170–14180.
[34] J. Wang, L. Yang, L. Wang, et al., Forkhead box p3 controls progression of oral lichen planus by regulating microRNA-146a, J. Cell. Biochem. 119 (2018) 8862–8871.
[35] L. Qin, F. Huimin, Q. Valerie, et al., Induced Foxp3(+) regulatory T cells: a potential new weapon to treat autoimmune and inflammatory diseases? J. Mol. Cell Biol. 4 (2012) 22–28.
[36] V. Vissia, B.A. Clare, H.L. Weiner, D.A. Hafler, Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis, J. Exp. Med.

199 (2004) 971–979.
[37] M. Kumar, N. Putzki, V. Limmroth, et al., CD4 + CD25 + FoxP3 + T lymphocytes fail to suppress myelin basic protein-induced proliferation in patients with multiple sclerosis, J. Neuroimmunol. 180 (2006) 178–184.
[38] V. Xavier, S. Geoffrey, G.M. Raphaela, et al., TNF downmodulates the function of human CD4 + CD25hi T-regulatory cells, Blood 108 (2006) 253–261.
[39] L. Li-Fan, M.P. Boldin, C. Ashutosh, et al., Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses, Cell 142 (2010) 914–929.
[40] H. Wang, D. Zhang, Q. Han, et al., Role of distinct CD4(+) T helper subset in pathogenesis of oral lichen planus, Journal of Oral Pathology & Medicine 45 (2016) 385–393.
[41] Jing W, Lijie Y, Luyao W et al. Forkhead box p3 controls progression of oral lichen planus by regulating microRNA-146a. J. Cell. Biochem..
[42] L. Runhua, Y. Bin, W. Shi, et al., FOXP3-miR-146-NF-κB Axis and therapy for precancerous lesions in prostate, Cancer Res. 75 (2015) 1714–1724.
[43] L. Runhua, L. Cong, C. Dongquan, et al., FOXP3 controls an miR-146/NF-κB negative feedback loop that inhibits apoptosis in breast cancer cells, Cancer Res. 75 (2015) 1703–1713.
[44] Z. Dunfang, W. Jiayi, L. Zhangwei, et al., The activation of NF-κB in infiltrated mononuclear cells negatively correlates with Treg cell frequency in oral lichen planus, Inflammation 38 (2015) 1683–1689.