RETRACTED ARTICLE: MicroRNA-210 protects against periodontitis through targeting HIF-3α and inhibiting p38MAPK/NF-κB pathway

Shuyu Jia*, Ximei Yang*, Xirong Yang and Fei Zhang
Department of Stomatology, Linyi Central Hospital, Shandong, China

ABSTRACT
The aim of this study was to investigate the effects of miR-210 abnormal expression on Porphyromonas gingivalis lipopolysaccharide (LPS)-treated primary human periodontal ligament cells (PDLCs). The miR-210 level was identified in gingival tissues from patients with chronic periodontitis (CP) and healthy subjects as well as LPS-treated PDLCs by qRT-PCR. Cell viability, apoptotic cells, expression of proteins associated with apoptosis, and release of inflammatory factors in LPS-treated PDLCs were measured using MTT assay, flow cytometry assay, western blotting and ELISA, respectively. Effects of miR-210 abnormal expression on cell viability, cell apoptosis and inflammation factors in LPS-treated PDLCs were evaluated. Afterwards, the target gene of miR-210 was identified, and the involvement of p38MAPK/NF-κB pathway with the effects of miR-210 was finally studied. The miR-210 level was significantly down-regulated in gingival tissues from CP patients as well as LPS-treated PDLCs. LPS-induced decrease of cell viability, increase of apoptosis, and release of TNF-α, IL-1β, IL-6 and IL-8 were attenuated by miR-210 overexpression. We found that hypoxia-inducible factor (HIF)-3α was a target of miR-210, and HIF-3α overexpression partly reversed the effects of miR-210 up-regulation on cell viability, cell apoptosis and inflammation factors expression in LPS-treated PDLCs. Moreover, the phosphorylation levels of key kinases in the NF-κB and p38MAPK pathways were reduced by miR-210 via targeting HIF-3α in LPS-treated PDLCs. MiR-210 attenuated LPS-induced periodontitis, and the LPS-induced activation of the NF-κB and p38MAPK pathways was attenuated by miR-210 via targeting HIF-3α in PDLCs.

Introduction
Periodontitis is a common and complex infectious disease that usually responds to subgingival plaque bacteria, such as Porphyromonas gingivalis (P. gingivalis), and affects a large population worldwide [1,2]. It is clinically characterised by gingival swelling and bleeding, alveolar bone osteopenia, periodontal pocket formation, loss of periodontal attachment, and ultimately teeth exfoliation [1,2]. Although conventional treatment methods such as cleaning, surgery, medicine, and fixed loose teeth have achieved considerable progress, the therapeutic effect is still unsatisfactory [3]. Therefore, exploring the molecular pathogenesis of periodontitis is imperative, which can provide a favourable theoretical basis for its treatment.

Studies have shown that multiple factors, including plaque microbes, calculus, oral hygiene, microbial infections, contribute to the development of periodontitis [4]. A large number of lymphocytes accumulate in the periodontal tissues of patients with periodontitis, which promotes the occurrence of periodontitis [5]. Plenty evidence indicates that the occurrence and development of periodontitis are significantly related to many molecular biomarkers, such as microRNA (miRNA) [6,7]. MiRNAs, as endogenous non-coding small-molecule RNAs, widely exist in severe conditions [8]. Previous studies have demonstrated that miRNA dysregulation remarkably influences the development of periodontitis, such as miR-146a [9], miR-128 [10], and miR-200b [11]. MiR-210 has been identified to be involved in the development of various diseases such as cancers [12] and immunological diseases [13,14]. Based on bioinformatics methods, scholars have predicted a variety of differentially expressed miRNAs involved in the development of periodontitis, of which miR-210 is shown to be lowly expressed in diseased gingival tissues [15]. However, few studies have investigated the relationship between the miR-210 level and the pathogenesis of periodontitis.

In the current study, primary human periodontal ligament cells (PDLCs) were separated and treated with P. gingivalis lipopolysaccharide (LPS) to induce periodontitis model. The miR-210 level was identified in patients with chronic periodontitis (CP) and LPS-treated PDLCs, and then the effect of miR-210 abnormal expression on LPS-treated PDLCs was investigated.
Materials and methods

Gingival sample collection
A total of 24 patients with CP and 18 healthy subjects were recruited from our hospital between November 2017 and November 2018. The gingival tissue samples from CP patients were collected during surgical therapy, and healthy gingival tissue samples from healthy subjects were obtained during crown-lengthening procedures. The ethical approval was obtained from the ethics committee of Linyi Central Hospital in this study.

Cell isolation, culture and treatment
Primary human periodontal ligament cells (PDLCs) were isolated from healthy periodontal ligament tissues at the one-third of the root of molars that removed during orthodontic treatment using the enzyme digestion method [16]. The cells were maintained in DMEM (Gibco, Carlsbad, CA, USA) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 20% foetal bovine serum (FBS, Gibco) with standard incubation conditions (5% CO2 and 37°C). The cells were maintained in DMEM (Gibco, Carlsbad, CA, USA) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 20% foetal bovine serum (FBS, Gibco) with standard incubation conditions (5% CO2 and 37°C). PDLCs in the third passage were utilised in the following experiments. For establishing periodontitis model, PDLCs were treated with 10 μg/ml P. gingivalis LPS (Sigma, St Louis, MI, USA).

Cell transfection
PDLCs were inoculated in six-well plates for 24 h with approximately 5 × 10⁵ cells in each well, and then commercial miR-210 mimic, mimic NC, inhibitor NC or miR-210 inhibitor (Thermo, Waltham, MA, USA) was transfected into LPS-treated PDLCs cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) as manufacturer’s instructions. Meanwhile, PDLCs without transfection served as a control group. In addition, to investigate the effect of hypoxia-inducible factor (HIF)-3α on LPS-induced PDLCs, the construction of HIF-3α overexpressed vector (pc-HIF-3α) was performed and empty vector (pcDNA-3.1) was provided by Biosyntech Co., Ltd (Suzhou, China). Then LPS-induced PDLCs were transfected with miR-210 mimic and pc-HIF-3α or pcDNA-3.1 by Lipofectamine 2000.

Quantitative real-time reverse transcription PCR (qRT-PCR)
Total RNA from cells was obtained by Trizol (Invitrogen, Gaithersburg, MD, USA), and then cDNA was obtained by reverse transcription of RNA using Mir-miRNA First-Strand Synthesis Kit or PrimeScript™ RT reagent Kit (Takara, Dalian, China). The RT-qPCR was performed by a SYBR Premix Ex Taq (Takara, Otsu, Shiga, Japan). The expression of target gene was detected using a BioTek microplate reader and was calculated using the ΔΔCt method.

MTT assay
PDLCs with different treatments were grown in 96-well plates. Then MTT (10 μL, 5 mg/mL) (Sigma) was added into each well every 24 h for 4 h, followed by the addition of 100 μL dimethyl sulfoxide (DMSO, Sigma). The zero hole (medium, MTT, DMSO) and blank hole were set up. The absorbances at 570 nm were read after incubation for 24,487,296 h by microplate reader (Molecular Devices, USA).

Enzyme-linked immunosorbent assay (ELISA)
The supernatants of PDLCs with different treatments were collected, and then determined by commercial ELISA kits (Boster, Wuhan, China) for the contents of tumour necrosis factor (TNF)-α, interleukins (IL)-1β, IL-6 and IL-8 according to the manufacturer’s instructions.

Apoptosis assay
Annexin V-FITC Apoptosis Detection kit was used to evaluate the cell apoptosis. The PDLCs with various treatments were digested with Trypsin. After washing with PBS, cells were resuspended with 1 × Binding Buffer, followed the incubation with PI and FITC-Annexin V for 15 min at 25°C in the dark, and then with 1 × Binding Buffer. Cells were finally detected using flow cytometer (BD, CA, USA).

Luciferase reporter assay
The target gene of miR-210 was predicted by TargetScan databases, and verified using the luciferase reporter assay. The 3‘-UTR-wild-type (WT) or mutant (MUT) of HIF-3α was cloned into a pmirGLO vector, named as HIF-3α-WT or HIF-3α-MUT. Then miR-210 mimics or mimics NC plasmid was co-transfected with either HIF-3α-WT or HIF-3α-MUT into 293 T cells, respectively, for 48 h. Lastly, the Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) was used to measure the relative luciferase activities.

Western blotting analysis
Total proteins were isolated by RIPA Lysis Buffer (Beyotime, Shanghai, China). Proteins concentrations were tested by bicinchoninic acid (BCA) kit (Beyotime). The protein sample was separated on SDS-PAGE gel, and transferred to polycrylaminide fluoride membranes, followed by the blockage with 5% non-fat milk for 1 h. Next, the membranes were probed with primary antibodies of HIF-3α, Bcl-2, Bax, pro-caspase-3, cleaved-caspase-3, pro-caspase-9, cleaved-caspase-9, IκBα, P-IκBα, P-IκBα, p65, P-p65, p38 MAPK, and P-p38 MAPK (1: 1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and β-actin (1: 1000, Beyotime) overnight at 4°C. Then, membranes were incubated with secondary antibody (1: 1000, Beyotime) for
2 h keeping in dark place at room temperature. GAPDH was used as the control protein. Enhanced chemiluminescence (ECL) Plus reagent (Beyotime) was used to image blots. The band quantification was performed using Image J software.

**Statistical analysis**

GraphPad Prism 6 software was used for data statistical analysis. Data were expressed as the mean ± SD and analysed by one-way ANOVA followed by multiple comparison with the Tukey test. A value of $p < .05$ was considered significant.

**Results**

**MiR-210 is decreased in patients with CP**

The miR-210 level was detected in gingival tissues from healthy subjects and CP patients, and the results showed significantly decreased miR-210 level in CP patients compared with healthy subjects ($p < .05$, Figure 1).

**MiR-210 is decreased in LPS-induced periodontitis**

PDLCs were treated with LPS and the results revealed that cell viability was significantly inhibited in time-dependent manner after LPS treatment compared with control cells ($p < .05$, Figure 2(A)). Cell apoptosis rate was remarkably increased in time-dependent manner in LPS-treated PDLCs compared with control cells ($p < .05$, Figure 2(B)). Consistently, western blotting showed that LPS distinctly inhibited Bcl-2 level as well as promoted the expression of Bax, cleaved-caspase-3, and cleaved-caspase-9 (Figure 2(C)). Moreover, LPS treatments promoted the concentrations of inflammatory factors, including TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-8, in time-dependent manner in comparison with control cells ($p < .05$, Figure 2(D)). Notably, miR-210 level was prominently reduced in time-dependent manner in LPS-treated PDLCs compared with control cells ($p < .05$, Figure 2(E)), which was consistent with clinical samples.

![Figure 1](image1.png)

**Figure 1.** The expression of miR-210 in patients with chronic periodontitis (CP). The miR-210 level in gingival tissues from healthy subjects and CP patients by qRT-PCR. *$p < .05$.*

![Figure 2](image2.png)

**Figure 2.** Effect of lipopolysaccharide (LPS) on cell viability, apoptosis and inflammatory response. (A) Cell viability of cells treated with LPS after 24, 48, 72 and 96 h in primary human periodontal ligament cells (PDLCs) by MTT assay. (B) The number of apoptotic cells of untreated cells, cells treated with LPS after 24, 48, 72 and 96 h in PDLCs by flow cytometry analysis. (C) The expression of apoptosis-related proteins (Bcl-2, Bax, pro-caspase-3, cleaved-caspase-3, pro-caspase-9, and cleaved-caspase-9) of untreated cells, cells treated with LPS after 24, 48, 72 and 96 h in PDLCs by western blotting. (D) The concentrations of inflammatory factors (TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-8) of untreated cells, cells treated with LPS after 24, 48, 72 and 96 h in PDLCs by enzyme-linked immunosorbent assay. (E) The miR-210 level of untreated cells, cells treated with LPS after 24, 48, 72 and 96 h in PDLCs by qRT-PCR. *$p < .05$, **$p < .01$, and ***$p < .001$. 
Effects of miR-210 abnormal expression on cell viability, cell apoptosis and cell inflammation factors

Firstly, compared with NC cells, miR-210 level was significantly up-regulated in PDLCs with miR-210 mimic, and remarkably down-regulated after transfection with miR-210 inhibitor ($p < .001$, Figure 3(A)). MTT assay showed that miR-210 knockdown obviously inhibited cell viability in time-dependent manner in LPS-treated PDLCs, while miR-210 over-expression exerted promoting effect on cell viability ($p < .05$,
Flow cytometry analysis found that the number of apoptotic cells was prominently decreased in LPS-treated PDLCs transfected with miR-210 mimic compared with LPS-treated PDLCs with mimic NC, meanwhile, miR-210 knockdown increased the number of apoptotic cells in LPS-treated PDLCs ($p < .01$, Figure 3(C)). Consistently, western blotting showed that miR-210 overexpression distinctly promoted Bcl-2 level as well as inhibited the expression of Bax, cleaved-caspase-3, and cleaved-caspase-9, while miR-210 knockdown had the opposite effects (Figure 3(D)). In addition, miR-210 overexpression obviously inhibited the concentrations of TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-8 in LPS-treated PDLCs, while miR-210 knockdown exerted promoting effect on the concentrations of inflammatory factors ($p < .05$, Figure 3(E)).

HIF-3$\alpha$ is identified as a target gene of miR-210

Based on TargetScan databases, HIF-3$\alpha$ was considered as a potential target gene of miR-210 (Figure 4(A)). Luciferase reporter assay showed that the relative luciferase activity was significantly reduced after co-transfection with miR-210 mimic and HIF-3$\alpha$-WT compared with co-transfection with miR-210 NC (Figure 4(B)), while significant difference was not found after HIF-3$\alpha$-MUT treatment, which suggested HIF-3$\alpha$ as a direct target gene of miR-210. In addition, the mRNA level of HIF-3$\alpha$ was obviously decreased in PDLCs with miR-210 mimic, and remarkably increased after transfection with miR-210 inhibitor ($p < .01$, Figure 4(C)), which was consistent with the protein expression of HIF-3$\alpha$ (Figure 4(D)).

Effects of miR-210 on LPS-induced PDLCs cell viability, cell apoptosis and cell inflammation factors are through regulation of HIF-3$\alpha$

Compared with healthy subjects, the mRNA levels of HIF-3$\alpha$ were both significantly increased in gingival tissues from CP patients ($p < .01$, Figure 5(A)). Consistently, LPS treatment obviously elevated the mRNA and protein levels of HIF-3$\alpha$ in time-dependent manner ($p < .05$, Figure 5(B,C)). HIF-3$\alpha$ expression was significantly up-regulated after transfection with pc-HIF-3$\alpha$ compared with pcDNA3.1 ($p < .001$, Figure 5(D,E)). Co-transfection of miR-210 mimic and pc-HIF-3$\alpha$ remarkably inhibited cell viability ($p < .5$, Figure 5(F)), promoted cell apoptosis ($p < .01$, Figure 5(G,H)), and increased the concentrations of TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-8 ($p < .01$, Figure 5(I)) compared with co-transfection of miR-210 mimic and pcDNA3.1 in LPS-treated PDLCs.

MiR-210 protects against LPS-induced PDLCs injury through inhibiting p38MAPK/NF-$\kappa$B pathway

LPS treatment significantly inhibited the phosphorylation level of I$\kappa$B$\alpha$ and increased the phosphorylation levels of p65 and p38 MAPK, while miR-210 overexpression reversed the effects of LPS on the phosphorylation level of I$\kappa$B$\alpha$, p65, and p38 MAPK, and further HIF-3$\alpha$ up-regulation recovered the phosphorylation level of I$\kappa$B$\alpha$ and p65 induced by miR-210 overexpression ($p < .05$, Figure 6).

Discussion

The present study found that the miR-210 level was significantly down-regulated in gingival tissues from CP patients as well as LPS-treated PDLCs. LPS treatment inhibited cell viability, induced cell apoptosis, and promoted inflammatory response in PDLCs; However, overexpressed miR-210 inhibited LPS-induced PDLCs injury. In addition, HIF-3$\alpha$ was identified as a target gene of miR-210, and HIF-3$\alpha$ overexpression partly reversed the effects of miR-210 up-regulation on cell viability, cell apoptosis and inflammation factors expression in LPS-treated PDLCs. Furthermore, p38MAPK/NF-$\kappa$B pathway...
Figure 5. Relationship of HIF-3α and the effects of miR-210 in lipopolysaccharide (LPS)-induced primary human periodontal ligament cells (PDLCs). (A) The mRNA level of HIF-3α in gingival tissues from healthy subjects and CP patients by qRT-PCR. (B) and (C) The mRNA and protein levels of HIF-3α of untreated cells, cells treated with lipopolysaccharide (LPS) after 24, 48, 72 and 96 h in PDLCs by qRT-PCR and western blotting. (D) and (E) The mRNA and protein levels of HIF-3α of cells treated with pcDNA3.1 and pc-HIF-3α in PDLCs by qRT-PCR and western blotting. (F) Cell viability of untreated cells, cells with LPS, LPS + mimic NC, LPS + miR-210 mimic, LPS + miR-210 mimic + pcDNA3.1, LPS + miR-210 mimic + pc-HIF-3α in PDLCs by MTT assay. (G) The number of apoptotic cells of untreated cells, cells with LPS, LPS + mimic NC, LPS + miR-210 mimic, LPS + miR-210 mimic + pcDNA3.1, LPS + miR-210 mimic + pc-HIF-3α in PDLCs by flow cytometry analysis. (H) The expression of apoptosis-related proteins (Bcl-2, Bax, pro-caspase-3, cleaved-caspase-3, pro-caspase-9, and cleaved-caspase-9) of untreated cells, cells with LPS, LPS + mimic NC, LPS + miR-210 mimic, LPS + miR-210 mimic + pcDNA3.1, LPS + miR-210 mimic + pc-HIF-3α in PDLCs by western blotting. (I) The concentrations of inflammatory factors (TNF-α, IL-1β, IL-6 and IL-8) of untreated cells, cells with LPS, LPS + mimic NC, LPS + miR-210 mimic, LPS + miR-210 mimic + pcDNA3.1, LPS + miR-210 mimic + pc-HIF-3α in PDLCs by enzyme-linked immunosorbent assay. *p < .05, **p < .01, and ***p < .001.
was revealed to be associated to the effects of miR-210 on LPS-treated PDLCs.

Accumulating evidence have indicated that miR-210 is a major miRNA induced under hypoxia [17]. It has been shown that miR-210 is generally elevated in several cancers such as glioblastoma [18], pancreatic cancer [19], and breast cancer [20], and exhibits oncogenic properties [12]. Interestingly, miR-210 level is reduced in immunological diseases, including rheumatoid arthritis [21], osteoarthritis [13], and Alzheimer’s disease [22]. Similarly, this study also demonstrated the down-regulated miR-210 level in gingival tissues from CP patients and LPS-treated PDLCs. This study further investigated the effects of miR-210 on cell viability, cell apoptosis and cell inflammation factors in LPS-treated PDLCs. Previous study has suggested that miR-210 overexpression could inhibit the expression of pro-inflammatory cytokines such as IL-6 and TNF-α induced by Toll-like receptor 4 [23]. Also, miR-210 is considered as a feedback negative regulator in inflammation response induced by LPS [23]. Moreover, study has demonstrated that miR-210 overexpression induces cell apoptosis and inhibits cell proliferation, thereby promoting cancer progress [24]. The results of this study revealed that miR-210 mimic exhibited anti-apoptotic and anti-inflammatory effects in LPS-induced PDLCs.

Extensive scholars have investigated miR-210 as one of the miRNAs regulated by hypoxia in cancer. It is well-known that hypoxia is a major characteristic in the tumour microenvironment [25], and exerts critical roles in cell proliferation, cell apoptosis, tumour invasion and metastasis, and angiogenesis [26]. Hypoxia has been widely confirmed as an independent adverse prognostic factor in cancers [25]. This study confirmed that HIF-3α was a target gene of miR-210, which was consistent with the study of Li et al. [27]. HIF-3α has been reported to be down-regulated in renal cell carcinoma, and is considered as a dominant-negative regulator of HIF-1 [28]. HIF-1 overexpression can inhibit cell apoptosis in cancers [29]. In this study, up-regulated HIF-3α inhibited cell viability, promoted cell apoptosis and inflammation factors expression in LPS-induced PDLCs, exerting contrary effect to miR-210. Previous study revealed that miR-210 can inhibit hypoxia-induced cell apoptosis by hypoxia pathway [30]. Thus, we speculated that the anti-apoptotic and anti-inflammatory effects of miR-210 in LPS-induced PDLCs might be regulated via targeting HIF-3α.

Furthermore, both p38MAPK and NF-κB signalling pathways have been demonstrated to be implicated in cancer progress and inflammatory response [31,32]. Recent study has shown that p38MAPK signalling pathway can be activated to induce the expression of pro-inflammatory cytokines in periodontitis [33]. NF-κB is also reported to be activated in LPS-induced PDLCs and periodontal diseases [34]. Noteworthily, Zhang et al. have suggested that miR-210 is able to inhibit NF-κB signalling pathway, thereby reducing inflammatory responses in osteoarthritis [13]. In the present study, the roles of p38MAPK and NF-κB signalling pathways were investigated in LPS-induced PDLCs. The present results revealed that LPS treatment activated both p38MAPK and NF-κB signalling pathways, while miR-210 overexpression inhibited p38MAPK/NF-κB pathway in LPS-induced PDLCs, which was similar to the report of Chen et al. [35]. These results indicated a significant association between miR-210 expression and p38MAPK/NF-κB pathway in LPS-induced PDLCs.

In conclusion, this study reported that miR-210 level was down-regulated in periodontitis, and miR-210 could attenuate LPS-induced cell apoptosis and inflammatory injury in PDLCs, possibly through targeting HIF-3α and inhibiting p38MAPK/NF-κB pathway. However, the results in our study should be further verified by animal experiments.

Disclosure statement
The authors declare that they have no conflicts of interest to disclose.

References
[1] Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15(1):30.
[2] Papapanou PN, Susin C. Periodontitis epidemiology: is periodontitis under-recognized, over-diagnosed, or both?. Periodontol 2000. 2017;75(1):45–51.
S. JIA ET AL.

[3] Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017;75(1):7–23.

[4] Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol. 2010;8(7):481.

[5] Kornman KS. Mapping the pathogenesis of periodontitis: a new look. J Periodontol. 2008;79(6):1560–1568.

[6] Xie Y, Shen R, Jiang S, et al. Comparison of microRNA profiles of human periodontal diseased and healthy gingival tissues. Int J Oral Sci. 2011;3(3):125.

[7] Du A, Zhao S, Wan L, et al. Micro RNA expression profile of human periodontal ligament cells under the influence of Porphyromonas gingivalis LPS. J Cell Mol Med. 2016;20(7):1329–1338.

[8] Acunzo M, Romano G, Wernicke D, et al. MicroRNA and cancer—a brief overview. Adv Biolog Regulat. 2015;57:1–9.

[9] Jiang S-Y, Xue D, Xie Y-F, et al. The negative feedback regulation of microRNA-146a in human periodontal ligament cells after Porphyromonas gingivalis lipopolysaccharide stimulation. Inflamm Res. 2015;64(6):441–451.

[10] Na HS, Park MH, Song YR, et al. Elevated MicroRNA-128 in periodontitis mitigates Tumor Necrosis Factor-α Response via p38 Signaling Pathway in Macrophages. J Periodontol. 2016;87(9):e173–e182.

[11] Kalea A, Hoteit R, Suvan J, et al. Upregulation of gingival tissue microRNA miR-200b in obese periodontitis subjects. J Dent Res. 2015;94(3_suppl):595–605.

[12] Dang K, Myers K. The role of hypoxia-induced miR-210 in cancer progression. Int J Mol Sci. 2015;16(12):6353–6372.

[13] Zhang D, Cao X, Li J, et al. MiR-210 inhibits NF-κB signaling pathway by targeting DR6 in osteoarthritis. Sci Rep. 2015;5(1):12775.

[14] Wang H, Flach H, Onizawa A, et al. Negative regulation of Hif-1α expression and T H 17 differentiation by the hypoxia-regulated microRNA-210. Nat Immunol. 2014;15(4):393.

[15] Stoecsklín-Wasmer C, Guarnieri P, Celenti R, et al. MicroRNAs and their target genes in gingival tissues. J Dent Res. 2012;91(10):934–940.

[16] Li C, Li C, Yue J, et al. miR-21 and miR-101 regulate PLAP-1 expression in periodontal ligament cells. Mol Med Rep. 2012;5(5):1340–1346.

[17] Baveloni A, Ramazzotti G, Poli A, et al. MiRNA-210: a current overview. Anticancer Res. 2017;37(12):6511–6521.

[18] Lai N, Wu D, Fang X, et al. Serum microRNA-210 as a potential noninvasive biomarker for the diagnosis and prognosis of glioma. Br J Cancer. 2015;112(7):1241.

[19] Greither T, Grochols LF, Udelnow A, et al. Elevated expression of microRNAs 155, 203, 210 and 222 in pancreatic tumors is associated with poorer survival. Int J Cancer. 2010;126(1):73–80.

[20] Camps C, Buffa FM, Colella S, et al. hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. Clin Cancer Res. 2008;14(5):1340–1348.

[21] Abdul-Maksoud R, Sediq A, Kattaia A, et al. Serum miR-210 and miR-155 expression levels as novel biomarkers for rheumatoid arthritis diagnosis. Br J Biomed Sci. 2017;74(4):209–213.

[22] Zhu Y, Li C, Sun A, et al. Quantification of microRNA-210 in the cerebrospinal fluid and serum: Implications for Alzheimer’s disease. Exp Ther Med. 2015;9(3):1013–1017.

[23] Qi J, Qiao Y, Wang P, et al. miR-210 negatively regulates LPS-induced production of proinflammatory cytokines by targeting NF-kB1 in murine macrophages. FEBS Lett. 2012;586(8):1201–1207.

[24] Li L, Huang K, You Y, et al. Hypoxia-induced miR-210 in epithelial ovarian cancer enhances cancer cell viability via promoting proliferation and inhibiting apoptosis. Int J Oncol. 2014;44(6):2111–2120.

[25] Vaupe1 P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev. 2007;26(2):225–239.

[26] Ruan K, Song G, Ouyang G, et al. Role of hypoxia in the hallmarks of human cancer. J Cell Biochem. 2009;107(6):1053–1062.

[27] Li Z, Meng D, Li G, et al. Overexpression of microRNA-210 promotes chondrocyte proliferation and extracellular matrix deposition by targeting HIF-3α in osteoarthritis. Mol Med Rep. 2016;13(3):2769–2776.

[28] Maynard MA, Evans AJ, Hosomi T, et al. Human HIF-3α is a dominant-negative regulator of HIF-1 and is down-regulated in renal cell carcinoma. FASEB J. 2005;19(11):1396–1406.

[29] Aldo P, Eksabetta C. Role of HIF-1 in Cancer Progression: Novel Insights. A review. Cur Mol Med. 2018;18:1–9.

[30] Qiu J, Zhou X-y, Zhou X-g, et al. Neuroprotective effects of microRNA-210 on hypoxic-ischemic encephalopathy. BioMed Res Int. 2013;2013:1.

[31] Gupta J, Nebreda AR. Roles of p38α mitogen-activated protein kinase in mouse models of inflammatory diseases and cancer. Febs J. 2015;282(10):1841–1857.

[32] Taniguchi K, Karin M. NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol. 2018;18(5):309.

[33] Huang W, Zhan Y, Zheng Y, et al. Up-regulated ferritin in periodontitis promotes inflammatory cytokine expression in human periodontal ligament cells through transferrin receptor via ERK/ P38 MAPK pathways. Clin Sci. 2019;133(1):135–148.

[34] Gözl L, Memmert S, Rath-Deschner B, et al. Hypoxia and P. gingivalis synergistically induce HIF-1 and NF-κB activation in PDL cells and periodontal diseases. Mediators of Inflamm. 2015;2015:1.

[35] Chen Y, Li H. Alkannin protects human renal proximal tubular epithelial cells from LPS-induced inflammatory injury by regulation of microRNA-210. Biomed Pharmacother. 2018;108:1679–1685.