IL-18 promotes the secretion of matrix metalloproteinases in human periodontal ligament fibroblasts by activating NF-κB signaling

FANG WANG[1], MIN GUAN[2], LITING WEI[3] and HUI YAN[1]

[1]Department of Stomatology, Tianjin Nankai Hospital, Tianjin 300010; [2]Department of Stomatology, Inner Mongolia Autonomous Region People's Hospital, Hohhot, Inner Mongolia 010017; [3]Department of Stomatology, Changchun Stomatological Hospital, Changchun, Jilin 130022, P.R. China

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Abstract. Chronic periodontitis is the most common periodontal disease and is characterized by progressive degeneration of periodontal tissue. Periodontal-specific pathogens can induce the expression of various inflammatory cytokines in periodontal ligament cells and their secretion into peripheral blood. These inflammatory cytokines have an important role in the occurrence and development of chronic periodontitis. ELISA was used to detect the expression of interleukin-18 (IL-18) protein in the serum and saliva of 30 healthy volunteers and 30 patients with chronic periodontitis. The clinical parameters that were assessed included plaque index, gingival index, periodontal probing depth and attachment loss. The effect of IL-18 on the viability of human periodontal ligament fibroblasts (hPDLFs) was examined using a Cell Counting Kit-8 assay. The effects of IL-18 on mRNA expression and secretion of matrix metalloproteinase (MMP)1, MMP2, MMP3 and MMP9 in hPDLFs were detected by reverse transcription-quantitative polymerase chain reaction and ELISA, respectively. The effect of IL-18 on the phosphorylation of nuclear factor-κB (NF-κB) p65 protein and the protein expression of MMP1, MMP2, MMP3 and MMP9 in hPDLF cells was detected by western blotting. The expression level of IL-18 in the serum of patients with chronic periodontitis was significantly higher than that of healthy volunteers, and the expression level of IL-18 in saliva was positively correlated with the periodontal destruction. However, IL-18 did not have a significant effect on the viability ability of hPDLFs. IL-18 promoted phosphorylation of NF-κB p65 protein in hPDLF, and increased the mRNA expression and protein secretion of MMP1, MMP2, MMP3 and MMP9. These findings indicate that IL-18 promotes the secretion of MMP1, MMP2, MMP3, and MMP9 in hPDLFs by activating the NF-κB signaling pathway, which has a key role in the development of chronic periodontitis. Therefore, targeting IL-18 may be a new research direction for the treatment of chronic periodontal disease.

Correspondence to: Dr Fang Wang, Department of Stomatology, Tianjin Nankai Hospital, 6 Changjiang Road, Nankai, Tianjin 300010, P.R. China
E-mail: shizhuojin1568@163.com

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Introduction

Oral tissue health is listed by the World Health Organization as one of the ten basic criteria for human health, and periodontal disease is one of the two major diseases of the oral cavity (1). Chronic periodontitis, which is a chronic inflammatory disease of the periodontium caused by microorganisms characterized by progressive destruction of the periodontium, is the most common type of periodontal disease (2,3). The main clinical manifestations of chronic periodontitis are swelling and bleeding of the gums, formation of periodontal pockets, loss of attachment, absorption of the alveolar bone, loosening of the teeth and, eventually, tooth loss. A variety of complex factors are involved in the pathogenesis of periodontitis, and the pathological process involves the interaction of complex microorganisms and host factors (4). The degree of disease progression and development is affected by the immune response of the host to bacterial invasion (5). Additionally, pathogenic endotoxins can induce the release of large amounts of inflammatory cytokines into the local and peripheral bloodstream. Numerous studies have reported the effects of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 and IL-8, in chronic periodontitis; but the role of IL-18 in the development and progression of chronic periodontitis remains unclear (6,7).

IL-18 is a complex, multi-functional cytokine that has an important regulatory role in immune and inflammatory responses, and is a sensitive marker of inflammation. IL-18 can induce the production of interferon-γ (IFN-γ), enhance the cytotoxicity mediated by the Fas cell surface death receptor (Fas)-Fas ligand (FasL) system, promote Th1 cell proliferation and Th1 type immune responses, and also promote the secretion of inflammatory cytokines and chemokines (8,9).

IL-18 has anti-infection, antitumor and anti-hypersensitivity effects, and is associated with the development of inflammatory lesions in tissues and the occurrence of autoimmune diseases. Previous studies have reported that binding of IL-18 to IL-18 receptor α induces the activation of nuclear factor-κB (NF-κB), with signal transduction similar to the IL-1 signaling pathway (10).

NF-κB is a pleiotropic transcription factor that regulates the expression of a large number of genes involved in immune and inflammatory responses. NF-κB is usually in dimer form, with the earliest discovered heterodimer formed by p65 and p50. This NF-κB heterodimer has the most extensive distribution and effects among different dimers (11). A variety of stimuli, including bacterial or viral infections, ionizing radiation and inflammatory cytokines, activate the NF-κB signaling pathway. Activated NF-κB translocates to the nucleus and binds to target genes to promote transcription (12). NF-κB is involved in gene transcription associated with cell proliferation, apoptosis, inflammation and immunity. Recent studies have demonstrated that NF-κB activation increases the expression of matrix metalloproteinases (MMPs), as there are specific NF-κB binding sites in the proximal regulatory region of MMP gene promoters (13).

MMPs are a family of Zn^{2+}-containing neutral proteolytic enzymes that degrade extracellular matrix (ECM). Human MMPs are classified as collagenases, gelatinases, matrix lysins, membrane-type MMPs and other MMPs (14,15). The direct effects of MMPs in periodontal tissue destruction and degradation are involved in the development of periodontitis. Periodontal tissue contains type I collagen and type III-VII collagen, which are the major matrix components of the gingiva, periodontal ligament and bone (16). Compared with healthy periodontal tissues, MMP levels in connective tissue and gingival crevicular fluid were significantly increased in samples from patients with periodontitis, and the collagen was changed in shape, quantity and type. For example, structural changes and deformation of collagen fiber bundles were present in tissues from early periodontitis (17,18). MMPs can cause destruction of connective tissue, loss of attachment and degradation of collagen, in which collagen fragments can stimulate or attract osteoclasts to cause alveolar bone absorption. Studies have reported that the activity of MMP1, MMP2, MMP3, MMP8 and MMP9 is increased in gingival crevicular fluid and saliva in periodontitis. Furthermore, with the increasing degree of gingival inflammation, MMP activity and expression was also increased (19,20). Therefore, the MMP pathway may be a key pathway involved in periodontal tissue destruction.

In this study, the expression of IL-18 in the serum of patients with chronic periodontitis was significantly elevated, and the expression level of IL-18 in saliva increased with the increasing degree of periodontal destruction. Stimulation of human periodontal ligament fibroblasts (hPDLFs) with IL-18 promoted the phosphorylation of NF-κB p65 protein, and IL-18 promoted mRNA expression and protein secretion of MMP1, MMP2, MMP3 and MMP9 via activation of NF-κB signaling. These results indicate that IL-18 can promote the secretion of MMPs in hPDLF by activating the NF-κB signaling pathway, and thus, affect the occurrence and development of chronic periodontitis. The current study aimed to explore the role and significance of hPDLF IL-18 expression in chronic periodontitis.

Materials and methods

Research objective. Patients with chronic periodontitis (n=30) treated at the Department of Stomatology, Tianjin Nankai Hospital (Tianjin, China) from June 2017 to December 2017 were included in the chronic periodontitis group (18 males and 12 females). The age ranged from 21-67 years and the average age was 43.7±19.4 years. The inclusion criteria were as follows: i) Having been diagnosed of chronic periodontitis according to the standards formulated by the committee of periodontal disease research; ii) having no oral or regional inflammation, including amygdalitis and pharyngitis; iii) having no immune disease, infectious diseases or other chronic diseases; and iv) having not taken in any antibiotics or immunosuppressants in the last three months. Patients selected for physical examination in the same hospital during the same period were the control group (n=30; 14 males and 16 females). The age range was 24-62 years, and the average age was 45.2±17.7 years in the control group. Exclusion criteria were as follows: i) Having chronic diseases; ii) having been administered with antibiotics; iii) having oral inflammation; and iv) being pregnant. Written informed consent was obtained from subjects, and the study was approved by the Ethics Committee of Tianjin Nankai Hospital.

Peripheral blood collection. For patients with chronic periodontitis included in this study, peripheral venous blood was collected in the early morning following fasting. The specific procedure was as follows: A disposable serum separator hose produced by BD Biosciences (San Jose, CA, USA) was used to collect ~3 ml peripheral venous blood; following serum separation, the samples were centrifuged at 4˚C, 1,000 x g for 10 min. The upper layer was collected and stored in a -80˚C refrigerator prior to analysis.

Saliva specimen collection. Samples of saliva from patients and control subjects were collected in the early morning with a cotton swab. Subjects were required to have an empty stomach in the 2 h prior to saliva collection; during this time, subjects could not drink water, alcohol or beverages, eat, smoke, exercise or have severe emotional fluctuations. Subjects were in a sitting position, leaning slightly forward and the cotton swab was dipped in acid to stimulate oral saliva secretion during collection. Saliva was collected in 10 ml RNase-free tubes after 1.5 min and the upper layer liquid was collected immediately after centrifuging at 4˚C and 1,000 x g for 15 min. The collected saliva supernatant was frozen in a -80˚C refrigerator prior to use.

Routine periodontal examination. A detailed record of all periodontal clinical indicators was obtained for all patients, including plaque index (PLI), gingival index (GI), periodontal probing depth (PD) and loss of attachment (AL). PLI=the total amount of dental plaque/the total number of teeth. GI: The gingiva around each tooth was examined with a blunt periodontal probe and was divided into 4 tooth surfaces; the average score was obtained and the total score was the

average score of all the analyzed teeth. PD: The depth of the gum pocket or periodontal pocket measured with a special periodontal probe. AL: Using the periodontal probe, 20-25 g of force was extended into the gingival crevicular groove; the attachment of connective tissue decreased; the depth of the exploration was >3 mm and the enamel bone boundary was detected. The degree of periodontitis was measured according to probing depth, gingival index, loss of attachment and X-ray films showing alveolar bone resorption.

hPDLF cell culture. Periodontal ligament tissue of the patients and control subjects were used in the present study. A third of the periodontal ligament tissue of the root of bicuspids removed due to the orthodontics was cut into small pieces of 1 mm^3 and transplanted into culture bottles with a bottom area of 25 cm^2 containing 10% thermal non-animalized fetal bovine serum. Primary culture of tissue was performed in Dulbecco's modified Eagle's medium (DMEM; 100 U/ml, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and streptomycin (100 μg/ml) under 55% humidity and an atmosphere of 5% CO_2 at 37˚C. When hPDLFs were fully confluent, they were passaged at a ratio of 1:3. Following the third passage, the cell culture medium was changed to a mineralization induction medium (phenol red DMEM, 10% heat non-immobilized fetal bovine serum (Dalian Meilunbio Biology Technology Co., Ltd., Dalian, China), 10^{-8} mol dexamethasone, 10 mmol β-glycerophosphate sodium, 50 μg/ml ascorbic acid) to differentiate hPDLF cells. When passaged to the fourth generation, cells ($1.5x10^4/cm^2$ density) were seeded into a 6-well plate. At 90% confluence, the serum-free mineralization-inducing medium was replaced by mineralization induction medium and hPDLFs were further incubated for 12 h to prepare for subsequent experiments.The total mRNA of hPDLF cells was extracted by the Trizol reagent (Invitrogen; Thermo Fisher Scientific, Inc.).

ELISA detection. Human IL-18 Quantikine ELISA kit (cat. no. DL180), Human MMP1 Quantikine ELISA kit (cat. no. DMP100), MMP2 Quantikine ELISA kit (cat. no. MMP200), MMP3 Quantikine ELISA kit (cat. no. SMP300) and MMP9 Quantikine ELISA kit (cat. no. DMP900) were used to detect IL-18 and IL-1 in serum and saliva supernatants, and the content of MMP1, MMP2, MMP3, MMP9 in hPDLFs, respectively (all from R&D Systems China Co., Ltd., Shanghai, China). The experimental operations were performed in accordance with the instructions of the kit manufacturer.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) detection. An ABI StepOne Plus Real-Time PCR System was used to perform RT-qPCR for MMP1, MMP2, MMP3, MMP9, tissue inhibitor of metalloproteinase (TIMP)-1, and TIMP-2 using RNA obtained from hPDLFs, in strict accordance with the instructions of the Takara SYBR Premix Ex TaqPCR Reaction kit (Takara Biotechnology Co., Ltd., Dalian, China). The primers used for qPCR were as follows: MMP1, forward 5'-TCTGGGGAAAACCTTTCG ACT-3' reverse, 5'-CACCAACGTATTCAAAAGCACAA-3'; MMP2, forward 5'-TGACTTTCTTGGATCGGGTCG-3', reverse, 5'-AAGCACCACAGATGACTG-3'; MMP3, forward 5'-AGTCTTCCAATCCTACTGTTGCT-3', reverse 5'-TCC CCGTCACCTCCAATCC-3'; MMP9, forward 5'-TGTACC GCTATGGTTACACTCG-3', reverse, 5'-GGCAGGGACAGT TGCTTCT-3'; TIMP-1, forward 5'-CTTCTGCAATTCCGA CCTCGT-3', reverse, 5'-ACGCTGGTATAAGGTGGTCTG-3'; TIMP-2, forward 5'-AAGCGGTCAGTGAGAAGGAAG-3', reverse, 5'-GGGGCCGTGTAGATAAACTCTAT-3'; GAPDH, forward 5'-TGTGGGCATCAATGGATTTGG-3', reverse, 5'-ACACCATGTATTCCGGGTCAAT-3'; and IL-18, forward 5'-CAAGGCTGGTCCATGCTCC-3' and reverse, 5'-TGCTAT CACTTCCTTTCTGTTGC-3'.

The PCR mixture (20 μl) comprised 10 μl SYBR Premix Ex Taq II, 0.5 μl ROX Reference Dye II, 2 μl cDNA templates, 0.5 μl upstream primers, 0.5 μl downstream primers, RNAase Free dH_2O 6.5 μl. The thermocycling conditions were: 95˚C, 15 min; 95˚C, 15 sec; 60˚C, 60 sec, 35 cycles. The $2^{-\Delta\Delta Cq}$ method (21) was used to quantify expression.

Western blot analysis. hPDLFs were pretreated with pyrrolidine dithiocarbamate (PDTC, an inhibitor of NF-κB; 100 nM) for 24 h, and then stimulated with hPDLF with IL-18 (10 ng/ml) for 24 h. In the control group, the PDTC was replaced with a solvent (dimethyl sulfoxide, 0.1%). Radioimmunoprecipitation assay buffer (P0013B, Beyotime Institute of Biotechnology, Haimen, China) was used to extract total protein from the cells. Protein concentration was determined using a bicinchoninic acid assay. Denatured protein (50 μg/well) was then loaded into 10% SDS-polyacrylamide gels, followed by separation. Constant voltage (80 V) was applied until the samples formed a line, then the voltage was adjusted to 120 V and electrophoresis continued. Following electrophoresis, the protein was transferred to a polyvinylidene difluoride membrane on ice at 0.25 mA for 2 h. Ponceau staining for 10 min was used to detect successful transfer. Following washing with TBS-Tween buffer, the membrane was incubated for 2 h at room temperature in 5% non-fat dry milk powder on a shaker; then, primary antibody was added (P65, 8242S, 1:2,000, Cell Signaling Technology, Inc., Danvers, MA, USA; p-P65: 3031S, 1:2,000, Cell Signaling Technology, Inc.; MMP1: ab137332, 1:2,000, Abcam, Cambridge, UK; MMP2: ab37150,1:2,000, Abcam; MMP3: ab52915, 1:2,000, Abcam; MMP9: ab73734, 1:2,000, Abcam; GAPDH: ab8245, 1:5,000, Abcam) and incubated overnight at 4˚C. After washing the membrane, goat anti-rabbit IgG-horseradish peroxidase secondary antibody (ab6721, 1:2,000, Abcam) was added and incubated for 1 h at room temperature. Western blots were exposed by enhanced chemiluminescence (P0018, Beyotime Institute of Biotechnology) and band gray values were analyzed using ImageJ software version 1.43 (National Institutes of Health, Bethesda, MD, USA). The results were expressed as the experimental gray value/GAPDH band optical density value, and the internal reference was GAPDH.

Cell Counting Kit-8 (CCK-8) viability assay. According the manufacturer's instructions of the CCK-8 kit (Invitrogen; Thermo Fisher Scientific, Inc.), cells were inoculated in a 96-well (3,000 cells, 100 μl) plate. The hPDLFs were stimulated with IL-18 (10 ng/ml for 24 h at 37˚C) when 50% confluence was attained. In the control group, hPDLFs were stimulated with PBS for 24 h. After 24 h, CCK 8 reagent was added in

Table I. Levels of IL-18 in saliva.

Group	n	IL-18 (pg/μl)	P-value
Periodontitis group	30	22.45±3.65	0.005
Normal group	30	15.81±2.18	

IL-18, interleukin-18.

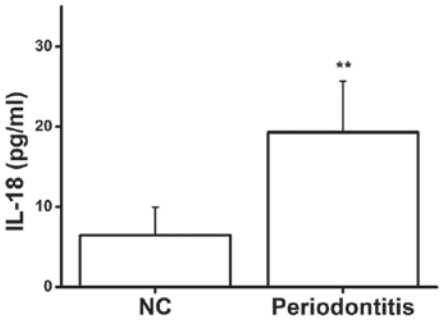

Figure 1. Expression of IL-18 protein in serum of patients with chronic periodontitis and normal subjects. **P<0.01 vs. NC. IL-18, interleukin-18; NC, normal control.

the medium, which accounted for 10% of the total volume The plate was then incubated for 4 h at 37˚C and 5% CO_2 incubator; 100 μl liquid was removed from each well and inserted into a 96-well enzyme-labeled plate, a chemiluminescent analyzer to determinate the absorbance values.

Statistical analysis. The results are presented as the mean ± standard deviation. Statistical analysis was performed using a Student's t-test. The correlation between variables was analyzed using linear regression analysis. All statistical analyses were performed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of IL-18 protein in serum of patients with chronic periodontitis. In this study, serum IL-18 protein levels in 30 healthy volunteers and 30 patients with chronic periodontitis were detected by ELISA. The serum IL-18 protein expression level in patients with chronic periodontitis was significantly higher than that in healthy volunteers (Fig. 1). This result suggests that there may be an association between IL-18 protein expression and chronic periodontitis.

Correlation of IL-18 expression in saliva with clinical parameters. Saliva specimens were collected from 30 patients with chronic periodontitis and IL-18 expression was detected in the saliva using ELISA (Table I). Additionally, clinical indicators of periodontitis patients were assessed and recorded, including PLI, GI, PD and AL. By performing linear regression analysis of the expression levels of IL-18 in saliva with these clinical indicators, it was determined that the expression levels of IL-18 in saliva were significantly positively correlated

Table II. Correlation of interleukin-18 expression in saliva with clinical parameters in patients with chronic periodontitis.

Clinical indicators	r	P-value
Plaque index	0.274	0.037
Gingival index	0.335	0.006
Periodontal probing depth	0.258	0.021
Loss of attachment	0.412	0.008

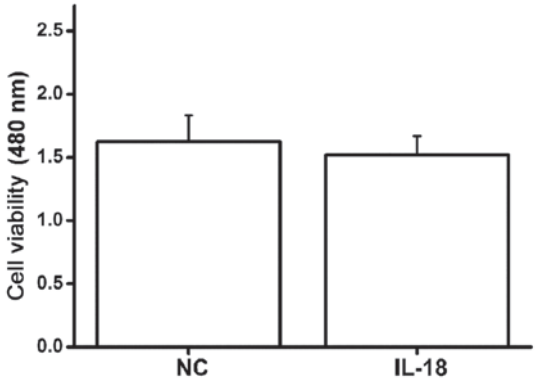

Figure 2. Effect of IL-18 on viability of human periodontal ligament fibroblasts. NC, negative control; IL-18, interleukin-18.

with each periodontal clinical index (Table II). This indicated that IL-18 expression is closely associated with periodontal destruction.

Effect of IL-18 on viability of hPDLFs. hPDLFs were stimulated with IL-18 (10 ng/ml) and the proliferation of cells was detected using CCK-8. After 24 h, the optical density value at 480 nm was detected and there was no significant difference between IL-18-stimulated cells and control cells (Fig. 2); thus, IL-18 had no effect on the viability of hPDLFs.

Effects of IL-18 on mRNA expression of MMP1, MMP2, MMP3 and MMP9 in hPDLFs. Following stimulation of hPDLFs with IL-18 (10 ng/ml) for 24 h, the cells were collected and the mRNA expression of MMPs in the IL-18-treated group and control group was detected by RT-qPCR. The mRNA expression levels of MMP1, MMP2, MMP3 and MMP9 were significantly increased in the IL-18-treated group compared with the controls (Fig. 3); however the mRNA expression levels of TIMP-1 and TIMP-2 did not change in the IL-18-treated group compared with the control group (Fig. 4). This indicated that IL-18 can promote the mRNA expression of MMP1, MMP2, MMP3 and MMP9 in hPDLFs, whereas it has no effect on TIMP-1 and TIMP-2.

Effect of IL-18 on MMP1, MMP2, MMP3 and MMP9 secretion in hPDLFs. To further investigate the effect of IL-18 on the protein expression levels of MMP1, MMP2, MMP3 and MMP9, ELISA was used to detect MMP protein levels in IL-18-stimulated (10 ng/ml) hPDLFs. MMP1, MMP2, MMP3 and MMP9 protein levels were significantly higher in the

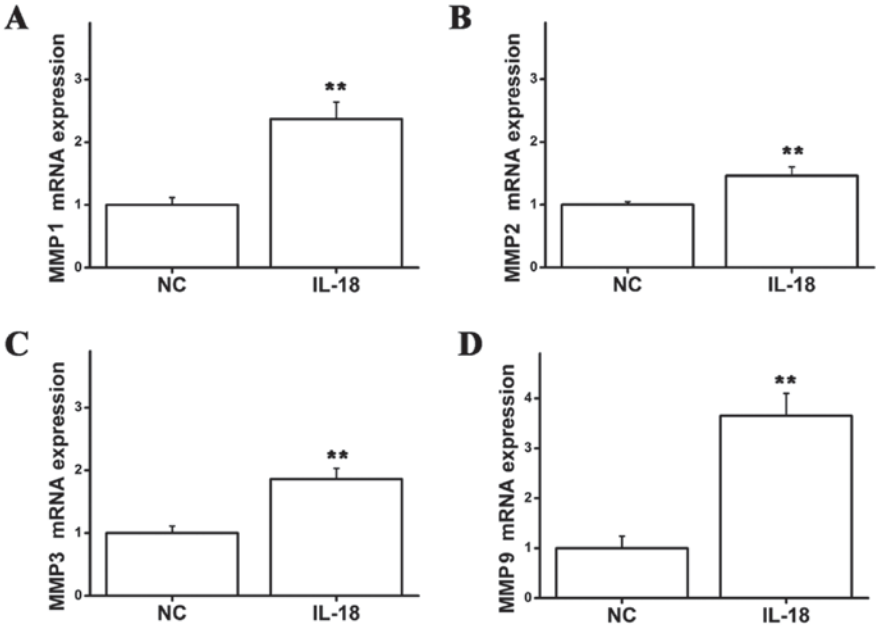

Figure 3. Effect of IL-18 on mRNA expression of MMP1, MMP2, MMP3 and MMP9 in human periodontal ligament fibroblasts. IL-18 promoted the mRNA expression of (A) MMP1, (B) MMP2, (C) MMP3 and (D) MMP9. **P<0.01 vs. NC. NC, negative control; IL-18, interleukin-18; MMP, matrix metalloproteinase.

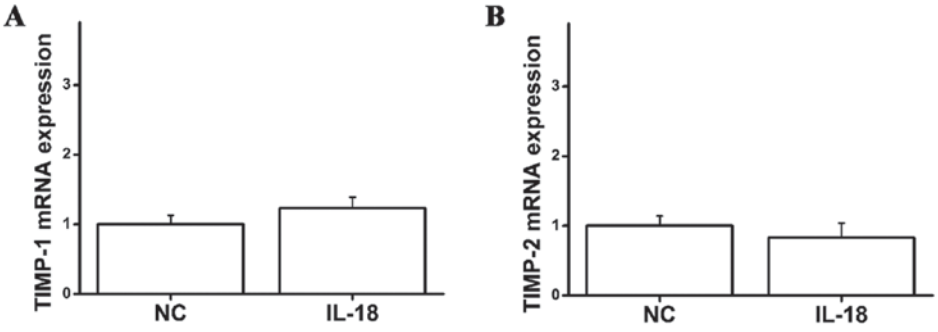

Figure 4. Effect of IL-18 on mRNA expression of TIMP-1 and TIMP-2. IL-18 treatment did not alter the expression of (A) TIMP-1 or (B) TIMP-2. NC, negative control; IL-18, interleukin-18; TIMP, tissue inhibitor of metalloproteinase.

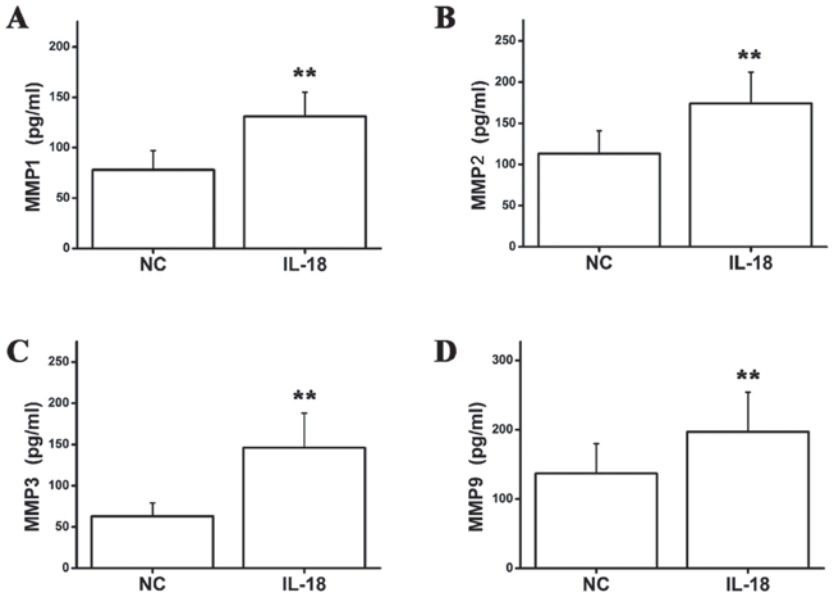

Figure 5. Effect of IL-18 on MMP1, MMP2, MMP3 and MMP9 protein secretion in hPDLF. IL-18 promoted the secretion of (A) MMP1, (B) MMP2, (C) MMP3 and (D) MMP9 protein. **P<0.01 vs. NC. NC, negative control; IL-18, interleukin-18; MMP, matrix metalloproteinase.

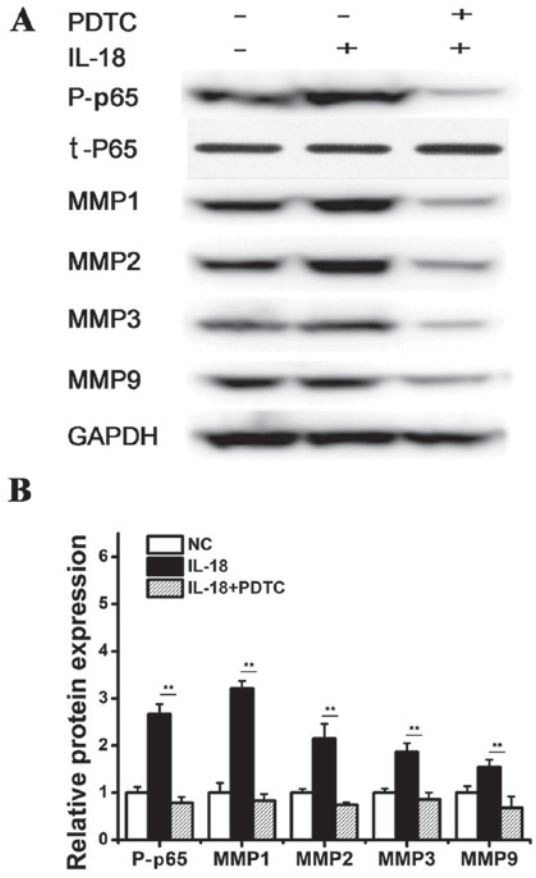

Figure 6. IL-18 regulates the expression of MMPs by activating the NF-κB pathway. (A) Western blotting analysis of MMPs expression and the phosphorylation of NF-κB p65 in different groups, and (B) semi-quantification of band density. **P<0.01. PDTC, pyrrolidine dithiocarbamate; IL-18, interleukin-18; P, phosphorylated; t, total; MMP, matrix metalloproteinase; NC, negative control, treated with dimethyl sulfoxide.

IL-18-treated group compared with the control group (Fig. 5). This result indicated that IL-18 promotes the secretion of MMP1, MMP2, MMP3 and MMP9 proteins by hPDLFs.

IL-18 regulates the expression of MMPs by activating the NF-κB pathway. Previous studies have demonstrated that NF-κB can promote the transcription of MMPs, and the activation of NF-κB can increase the expression of MMP1, MMP2, MMP3 and MMP9 (22). Therefore, this study examined whether IL-18 can promote the phosphorylation of NF-κB p65 protein to regulate the expression of MMPs. hPDLFs were pretreated with pyrrolidine PDTC, an inhibitor of NF-κB, and then stimulated with hPDLF with IL-18 (10 ng/ml). After 12 h, western blot analysis was performed to detect NF-κB. The phosphorylation NF-κB p65 protein was significantly increased in the IL-18-treated group, and the expression levels of MMP1, MMP2, MMP3 and MMP9 protein were also significantly increased compared with the control. However, the phosphorylation of NF-κB p65 was significantly decreased in the IL-18 + PDTC-treated group, and the protein expression levels of MMP1, MMP2, MMP3 and MMP9 were also suppressed compared with IL-18 treatment (Fig. 6). These results suggested that IL-18 induces the expression of MMP1, MMP2, MMP3 and MMP9 by activating the NF-κB signaling pathway.

Discussion

Periodontitis is a chronic infectious disease caused by microorganisms in dental plaque and is characterized by the progressive destruction of periodontal tissues (23). The endotoxins of periodontal-specific pathogens, including *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemura*, can cause a large number of inflammatory cytokines to be released in the local and peripheral blood system (24). Several studies have demonstrated that inflammatory cytokines, including TNF-α, IL-1β, IL-6 and IL-8, have important roles in the development of periodontal disease, and are associated with the development of chronic periodontitis. However, the association between IL-18 and chronic periodontitis is not yet well understood (25).

IL-18, a complex, multifunctional cytokine, is part of the IL-1 family and can be secreted by macrophages, endothelial cells, smooth muscle cells and other cell types. IL-18 promotes the production of IFN-γ by stimulation of T cells and natural killer cells, and enhances the Fas-FasL system-mediated cytotoxicity. IL-18 can also regulate the development and differentiation of Th1 cells, and promote Th1 cells to secrete various cytokines. It has been reported that IL-18 promotes the expression of various inflammatory factors and chemokines, including granulocyte-macrophage colony-stimulating factor, TNF-α, IL-1β and IL-2, which also contribute to various inflammatory diseases (26,27). In this study, serum IL-18 levels were detected in patients with chronic periodontitis; serum levels of IL-18 were significantly elevated in patients with chronic periodontitis compared with healthy volunteers, suggesting that IL-18 may be closely associated with chronic periodontitis. Furthermore, IL-18 expression was positively correlated with PLI, GI, PD and AL, indicating that IL-18 may have a pivotal role in periodontal destruction (28,29).

The key to regeneration of periodontal tissues is the proliferation and migration of residual periodontal ligament cells, and differentiation into osteoblasts and cementoblasts. hPDLFs are the major cellular component required for the development and function of periodontal tissues (30,31). hPDLFs have various functions, including chemotaxis, hyperplasia, adhesion, differentiation into cementoblasts and osteoblasts, and mineralized tissue formation. The proliferation, migration and differentiation of hPDLFs are crucial for regeneration of periodontal tissues (32). In this study, IL-18 did not have a significant functional effect on the proliferation of hPDLF, providing evidence to demonstrate that IL-18 is not involved in the development of chronic periodontitis via hPDLF proliferation.

MMPs are a family of proteolytic enzymes that degrade ECM and basement membrane components, with roles in remodeling, inflammation and other physiological and pathological processes involving connective tissue (33,34). MMPs have a crucial role in the degeneration of periodontal tissues in periodontitis. MMP1 is a collagenase that can degrade collagen I, II and III, and other ECM components, and is expressed at high levels in periodontal tissues (35). MMP2 is a gelatinase that can degrade gelatin and collagen I, II and III, and is involved in periodontal tissue destruction and inflammation (36). Another gelatinase, MMP9, is closely associated with periodontal tissue destruction in chronic periodontitis.

The expression level of MMP9 in gingival tissue, saliva and gingival crevicular fluid of patients with chronic periodontitis is significantly higher than that of healthy individuals, and therefore, could potentially be used as a clinical marker during the development of periodontitis (37). MMP3 is a matrix lysin that predominantly degrades type IV collagen, laminin and activates other MMPs, which further aggravates inflammation (38). Functional activities of MMPs are regulated by TIMPs. TIMPs participate in ECM maintenance in periodontal tissues by inhibiting the activity of MMPs. The levels of TIMPs are typically higher in healthy periodontal tissues than in inflammatory periodontal tissues; and in inflammatory periodontal tissues, the levels of MMPs are higher than the levels of TIMPs. The more severe the inflammation, the higher the concentration of active MMPs is (39,40). In a previous study, MMP1, MMP2, MMP3 and MMP9 were significantly higher in gingival crevicular fluid and gingival tissue of patients with periodontitis compared with in healthy individuals, while TIMP-1 and TIMP-2 levels were significantly decreased (41). In the present study, IL-18 was used to stimulate hPDLFs, and the mRNA expression of MMPs was subsequently determined by RT-qPCR. IL-18 increased the mRNA expression of MMP1, MMP2, MMP3 and MMP9 in hPDLFs; but notably, IL-18 did not affect the mRNA expression of TIMP-1 and TIMP-2. ELISA data revealed that IL-18 can increase the protein levels of MMP1, MMP2, MMP3 and MMP9 in hPDLFs. This suggests that IL-18 may promote the development of chronic periodontitis by upregulating the expression of MMP1, MMP2, MMP3 and MMP9.

NF-κB is a transcription factor with multi-directional regulatory functions. NF-κB can regulate the expression of IL-2, IL-6, IL-8, TNF-α, MMPs, granulocyte-colony stimulating factor and a series of other cytokines, and it is a pivotal transcriptional regulator of various immune and inflammation-associated genes (42). Burrage *et al* (43) reported that the promoter region of MMP1 gene contained an NF-κB binding site, in which NF-κB could bind to and initiate MMP1 transcription. There is also an NF-κB binding site at the -600 locus of MMP9. Andela *et al* (44) reported that blocking the NF-κB signaling pathway can downregulate the expression of MMP9. Rangaswami *et al* (45) reported that NF-κB has an important role in regulating the activity of MMP2 and MMP9. In this study, IL-18 was used to stimulate hPDLFs for 12 h, which significantly increased the phosphorylation of NF-κB p65 and increased the expression of MMP1, MMP2, MMP3 and MMP9 proteins. Additionally, hPDLF cells were pretreated with NF-κB inhibitor PDTC and then stimulated with IL-18, which resulted in significantly decreased phosphorylation of NF-κB p65 and decreased expression of MMP1, MMP2, MMP3 and MMP9 compared with the IL-18 treatment group. The results indicate that IL-18 can activate the NF-κB signaling pathway, and increase the expression of MMP1, MMP2, MMP3 and MMP9 via NF-κB activation.

In summary, IL-18 may have an important role in the development of chronic periodontitis. The data in the current study indicate that IL-18 can activate NF-κB signaling to promote the secretion of MMP1, MMP2, MMP3 and MMP9 by hPDLFs, which may have a role in promoting the development of chronic periodontitis. Therefore, further investigation of IL-18 may be an important research direction, and provide a therapeutic target for the diagnosis and treatment of chronic periodontitis.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FW designed the study, performed the experiments, analyzed the data and wrote the manuscript. MG, LW and HY performed the experiments. FW and MG analyzed the data, drafted and edited the manuscript, and designed and supervised the study.

Ethics approval and consent to participate

Written informed consent was obtained from subjects, and the study was approved by the Ethics Committee of Tianjin Nankai Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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