Expression of β-catenin and MMP-8 in gingival crevicular fluid and gingival tissue indicates the disease severity of patients with chronic periodontitis

LILEI ZHU[1], YAO YAO[2], JIAN LIU[1], JINGYU WANG[3] and HUI XIE[1]

Departments of [1]Periodontology, [2]Orthodontics and [3]Endodontics, Changsha Stomatological Hospital, Changsha, Hunan 410005, P.R. China

Received October 16, 2018; Accepted June 6, 2019

DOI: 10.3892/etm.2019.7794

Abstract. The aim of the present study was to investigate the interaction among β-catenin, matrix metalloproteinase-8 (MMP-8) and severity in patients with chronic periodontitis. Both gingival crevicular fluid (GCF) and gingival tissue was collected from 21 healthy control individuals, 21 patients with moderate chronic periodontitis (mCP) and 23 patients with severe chronic periodontitis (sCP). The concentration of MMP-8 in GCF was detected via ELISA and the mRNA levels of β-catenin and MMP-8 in GCF and gingival tissue was detected via reverse transcription-quantitative PCR. The protein levels of β-catenin and MMP-8 in gingival tissue was detected using western blotting and the interaction between β-catenin and MMP-8 in gingival tissue was detected by co-immunoprecipitation. The expression of β-catenin and MMP-8 was significantly higher in the GCF and gingival tissue of patients with chronic periodontitis (mCP and sCP) compared with the control patients. Furthermore, the expression of β-catenin and MMP-8 in GCF and gingival tissue was positively correlated with the clinical attachment level. In addition, a positive interaction was identified between β-catenin and MMP-8, and the expression of β-catenin was positively correlated with the expression of MMP-8 in GCF and gingival tissue. The CGF and gingival tissue expression of β-catenin and MMP-8 may indicate disease severity in patients with chronic periodontitis.

Introduction

Periodontitis is a bacteria-induced, chronic inflammatory disease that is characterized by the destruction of tooth-supporting tissues, the loss of periodontal attachment and the loss of bone, which affects 743 million individuals worldwide and represents the sixth most prevalent condition (1,2). Although currently available antibiotic therapies can reduce periodontal destruction, it is difficult to completely treat periodontal pathogens due to the complex anatomy of the furca area, pocket depth, the penetration of microorganisms into tissues and bacterial resistance (3). Additionally, surgical therapy is only available to a minority of individuals with periodontitis. Consequently, patients who undergo anti-periodontitis treatment are at an increased risk of future episodes of the disease, typically affecting the same sites (4). Therefore, it is necessary to understand and study the molecular mechanism underlying the disease to identify novel potential targets.

Previous studies have indicated that the degradation of the extracellular matrix (ECM) is one of the first stages of tooth-supporting tissue destruction (5,6). Matrix metalloproteinases (MMPs) are a family of structurally associated zinc-dependent proteolytic enzymes, which serve an essential role in ECM degradation, particularly MMP-8 (7,8). Previous studies have demonstrated that salivary MMP-8 levels are closely associated with the progressive loss of attachment in patients with periodontitis (8,9). A cross-sectional study indicated that the concentration of MMP-8 in the saliva of patients with severe periodontitis was increased, indicating that MMP-8 may serve as a biomarker of periodontal disease in larger patient populations (10). When oral bacteria stimulate periodontal tissues, elevated levels of MMPs, such as MMP-8, are closely associated with inflammatory and immune responses, which may accelerate ECM degradation and the destruction of the tooth-supporting tissues (11).

The Wnt/β-catenin signaling pathway is a well-conserved and well-studied pathway that coordinates stem cell maintenance, proliferation and cell-fate decisions during embryonic development and in adult tissue homeostasis (12). In the absence of Wnt ligands, β-catenin is normally localized in the cytoplasm and constantly degraded by a destruction complex, which is composed of adenomatous polyposis coli (APC), Axin, casein kinase-1α (CK-1α) and glycogen synthase kinase-3β (GSK-3β). When stimulated by Wnt ligands, a β-Catenin/APC/Axin/CK-1α/GSK-3β complex forms to promote the stabilization and translocation of β-catenin to

Correspondence to: Dr Hui Xie, Department of Periodontology, Changsha Stomatological Hospital, 389 Youyi Road, Changsha, Hunan 410005, P.R. China
E-mail: huixie2018@163.com

Key words: β-catenin, matrix metalloproteinase-8, gingival crevicular fluid, gingival tissues, chronic periodontitis

the nucleus, where it regulates T-cell factor (TCF)/lymphoid enhancer factor (LEF)-mediated gene transcription, including genes encoding ECM components (12-15). For example, Li *et al* (16) and Doyle *et al* (17) demonstrated that the accumulation of nuclear β-catenin upregulated the expression of MMP-7 and MMP-2. Additionally, several studies have confirmed the association between MMPs and periodontal diseases (18,19). Significantly higher levels of MMPs were reported in the saliva and gingival crevicular fluid (GCF) of patients with periodontitis compared with healthy individuals (20-22).

In summary, in regard to the central role of β-catenin and MMP-8 in ECM, it has been hypothesized that the expression of β-catenin and MMP-8 reflects the severity of chronic periodontitis. The aim of the present study was to investigate the association between β-catenin, MMP-8 and the severity of chronic periodontitis.

Materials and methods

Participants and clinical examination. A total of 65 adults who received medical treatment or physical examination at The Changsha Stomatological Hospital (Changsha, China) between 2015 and 2018 were recruited in the present study. All participants did not have a personal history of systemic diseases, such as coronary heart disease, hypertension and diabetes mellitus, and had not taken any medication (particularly antibiotics) for the preceding 6 months. Written informed consent was obtained from all participants or their lineal relatives. A total of 21 subjects were included in healthy group (8 females, 13 males; mean age, 36.90±2.02 years; age range, 22-52 years). Healthy subjects were free of periodontal diseases and had sites with <2 mm clinical attachment level (CAL), <3 mm probing depth (PD) and a bleeding on probing (BOP) score <15%. A further 44 subjects were diagnosed with chronic periodontitis according to the diagnostic criteria defined by the International Workshop for Classification of Periodontal Diseases and Conditions for Chronic Periodontitis (23). Patients with chronic periodontitis were classified into two groups according to the degree of CAL exhibited: Moderate chronic periodontitis (mCP; n=21; 12 females, 9 males; man age, 35.90±1.84 years; age range, 26-51 years) and severe chronic periodontitis (sCP; n=23; 10 females, 13 males; mean age, 36.78±1.71 years; age range, 28-51 years). Patients with mCP had at least three teeth exhibiting ≥3 and ≤5 mm CAL in at least two different quadrants. Patients with sCP had at least three teeth exhibiting >5 mm CAL in at least two different quadrants. The PD (24), CAL (24), plaque index (PI) (25) and BOP (26) were determined at six sites per tooth excluding the third molars. The measurements of PD (mm) and CAL (mm) were conducted using a Manual William's periodontal probe (Hu-Friedy Mfg., Co., LLC). The present study protocol was approved by The Ethics Committee of Changsha Stomatological hospital (Changsha, China).

Sample collection. All GCF samples of control, mCP and sCP patients were collected as during the initial clinical examination, prior to any treatment and/or hygiene procedures, as described previously (27,28). Subsequent to the removal of the supragingival plaque from interproximal surfaces using a sterile curette, surfaces were dried using an air syringe and isolated with cotton rolls. GCF was collected by placing filter paper strips (Periopaper; Harco Equipment Inc.) into the site with the deepest periodontal pocket until a slight resistance was felt, at which point strips were left in place for 30 sec. Strips contaminated with blood were excluded. Paper strips from each subject were pooled into an Eppendorf tube containing 1 ml PBS. Filter papers were eluted at room temperature for 40 min without shaking and centrifuged at 3,000 x g for 5 min at 4˚C, after which the supernatant was collected and immediately frozen at -20˚C until further analysis. Gingival tissue samples were collected from control, mCP and sCP patients prior to any periodontal treatment procedures. Gingival tissue samples of patients with mCP or sCP were collected from deep (>6 mm) periodontitis pockets via surgical incision at the bottom of the pocket. Teeth affected by severe and progressive periodontitis that were selected for the present study required extraction. Control specimens were collected during impacted third molar extraction surgery according to previous studies (29,30).

ELISA. The levels of MMP-8 in GCF samples from the control, mCP and sCP patients were determined using an ELISA kit (cat. no. DMP800B; R&D Systems, Inc.) according to the manufacturer's protocol.

Reverse transcription-quantitative PCR (RT-qPCR). The mRNA levels of β-catenin and MMP-8 in GCF and gingival tissue samples from the different groups were determined using RT-qPCR. Total RNA was extracted from human gingival tissue samples using TRIzol® (Invitrogen; Thermo, Fisher Scientific, Inc.). Total RNA was subsequently reverse transcribed into cDNA at 42˚C for 30-60 min using an Applied Biosystems Veriti-Well Thermal Cycler (Thermo Fisher Scientific, Inc.) using a PrimeScript RT reagent kit with genomic DNA Eraser (Takara Bio, Inc.). qPCR was performed using a SYBR green PCR kit (Takara Bio, Inc.) according to the manufacturer's protocol. Each PCR reaction contained 2 μl cDNA, 0.4 μl forward primer, 0.4 μl reverse primer, 7.2 μl H_2O_2 and 10 μl SYBR green. The amplification conditions were as follows: Initial denaturation at 95˚C for 15 min; 35 cycles of denaturation at 95˚C for 15 sec, annealing at 58˚C for 15 sec and extension at 72˚C for 30 sec. Experimental quantification cycle values were normalized to GAPDH, and the relative gene expression levels were determined using the $2^{-\Delta\Delta Cq}$ method (31). The primer sequences were as follows (all 5'-3'): β-catenin forward, CTCTGAGAAACTTGTCCGATG and reverse, GTGACCACATTTATATCATCAGAAC; MMP-8 forward, AGTGCCTGACAGTGGTGGTT and reverse, TCCCTGTGAGATCCTGGTGA; GAPDH forward, GCACCGTCAAGGCTGAGAAC and reverse, TGGTGAAGACGCCAGTGGA.

Western blotting. The protein expression of β-catenin and MMP-8 in GCF and gingival tissue samples from the different groups were determined using western blotting. Total protein in GCF samples were boiled in non-reducing Laemmli's buffer (Bio-Rad Laboratories, Inc.) whereas the total protein in gingival tissue was extracted using whole-cell protein extraction kits (Nanjing KeyGen Biotech Co., Ltd.). Total protein

Table I. Demographics and periodontal parameters.

Variable	Control (n=21)	mCP (n=21)	sCP (n=23)
Sex			
Male	8	12	10
Female	13	9	13
Age (years)	36.90±2.02	35.90±1.84	36.78±1.71
PD (mm)	1.79±0.46	2.58±0.45[a]	3.73±0.45[a,b]
CAL (mm)	0.19±0.30	3.89±0.56[a]	5.81±0.47[a,b]
PI (%)	8.47±1.97	76.52±5.90[a]	53.70±5.73[a,b]
BOP (%)	5.52±0.02	57.24±6.16[a]	44.59±4.68[a,b]

[a]P<0.05 vs. the control group; [b]P<0.05 vs. the mCP group. F, female; M, male; PD, probing depth; CAL, clinical attachment level; PI, plaque index; BOP, bleeding on probing; mCP, moderate chronic periodontitis; sCP, severe chronic periodontitis.

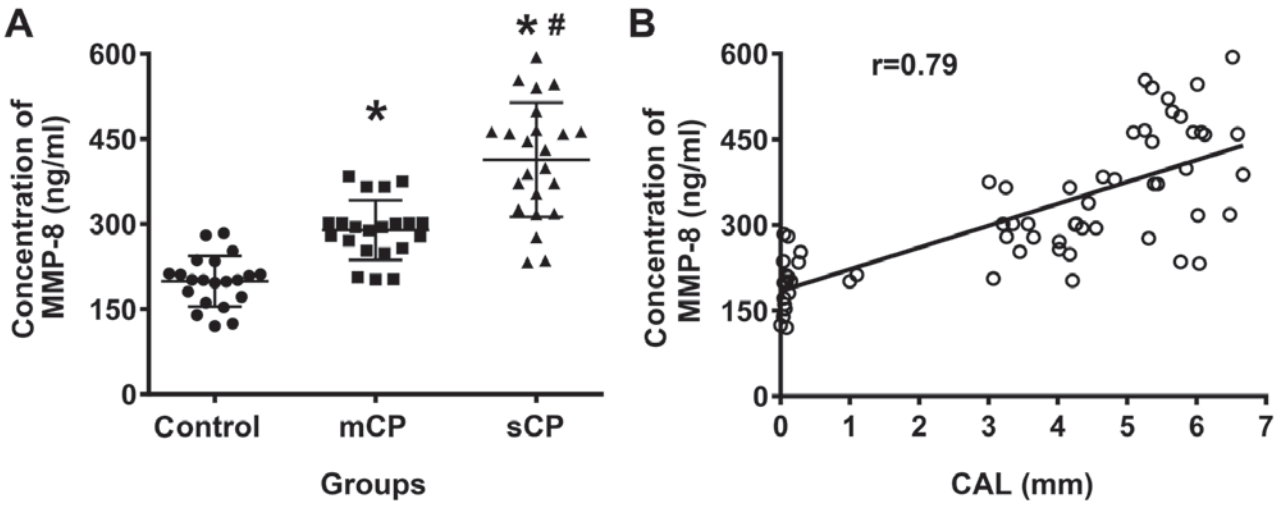

Figure 1. Concentration of MMP-8 in GCF. (A) Concentration of MMP-8 in GCF, as determined via ELISA. (B) Spearman's rank correlation analysis demonstrated a positive correlation between MMP-8 and CAL (r=0.79). *P<0.05 vs. the control group; #P<0.05 vs. the mCP group. MMP-8, matrix metalloproteinase; GCF, gingival crevicular fluid; mCP, moderate chronic periodontitis; sCP, severe chronic periodontitis; CAL, clinical attachment level.

concentration was determined using an enhanced bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. The proteins (20 μg/lane) were separated by 10% SDS-PAGE and transferred onto an Immobilon PVDF membrane (Millipore). After transfer, PVDF membranes were blocked with 5% non-fat milk for 1 h at 37˚C and subsequently incubated with the following primary antibodies overnight at 4˚C: Rabbit anti-MMP-8 (1:1,000; Abcam; cat. no. ab53017), rabbit anti-β-catenin (1:800; Abcam; cat. no. ab32572) and rabbit anti-GAPDH (1:4,000; Abcam; cat. no. ab181602). Subsequently, PVDF membranes were washed with TBS-Tween three times and incubated with a horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG; 1:2,000; Beyotime Institute of Biotechnology; cat. no. A0208) antibody for 2 h at room temperature. The signals were visualized using enhanced chemiluminescent Plus (Beyotime Institute of Biotechnology) and analyzed by Fusion FX5 image analysis system (Vilber Lourmat).

Co-immunoprecipitation. The interaction between β-catenin and MMP-8 in gingival tissue samples of the sCP group was measured by performing co-immunoprecipitation using protein A + G agarose (Beyotime Institute of Biotechnology) in accordance with the manufacturer's protocol. Total protein in gingival tissue samples was extracted using whole-cell protein extraction kit (Nanjing KeyGen Biotech Co., Ltd.). The harvested protein was incubated on a laboratory rocker with 4 μl of anti-β-catenin (Abcam; ab32572), anti-MMP-8 (Abcam; ab53017) or anti-IgG (Abcam; ab172730) at 4˚C for overnight. Then, samples were incubated with 40 μl protein A + G agarose at 4˚C for 4 h. Samples were then centrifuged at 1,000 x g and 4˚C for 5 min and the supernatant was discarded. The precipitate was washed using RIPA buffer (Beyotime Institute of Biotechnology), centrifuged at 1,000 x g and 4˚C for 5 min and supernatant was discarded. Then 200 μl of SDS-PAGE sample loading buffer (Beyotime Institute of Biotechnology) was added into the precipitate and the samples were boiled for 5 min. Subsequently, the samples was analyzed via western blotting as described above.

Statistical analysis. Data are expressed as the mean ± standard deviation of at least three independent experiments.

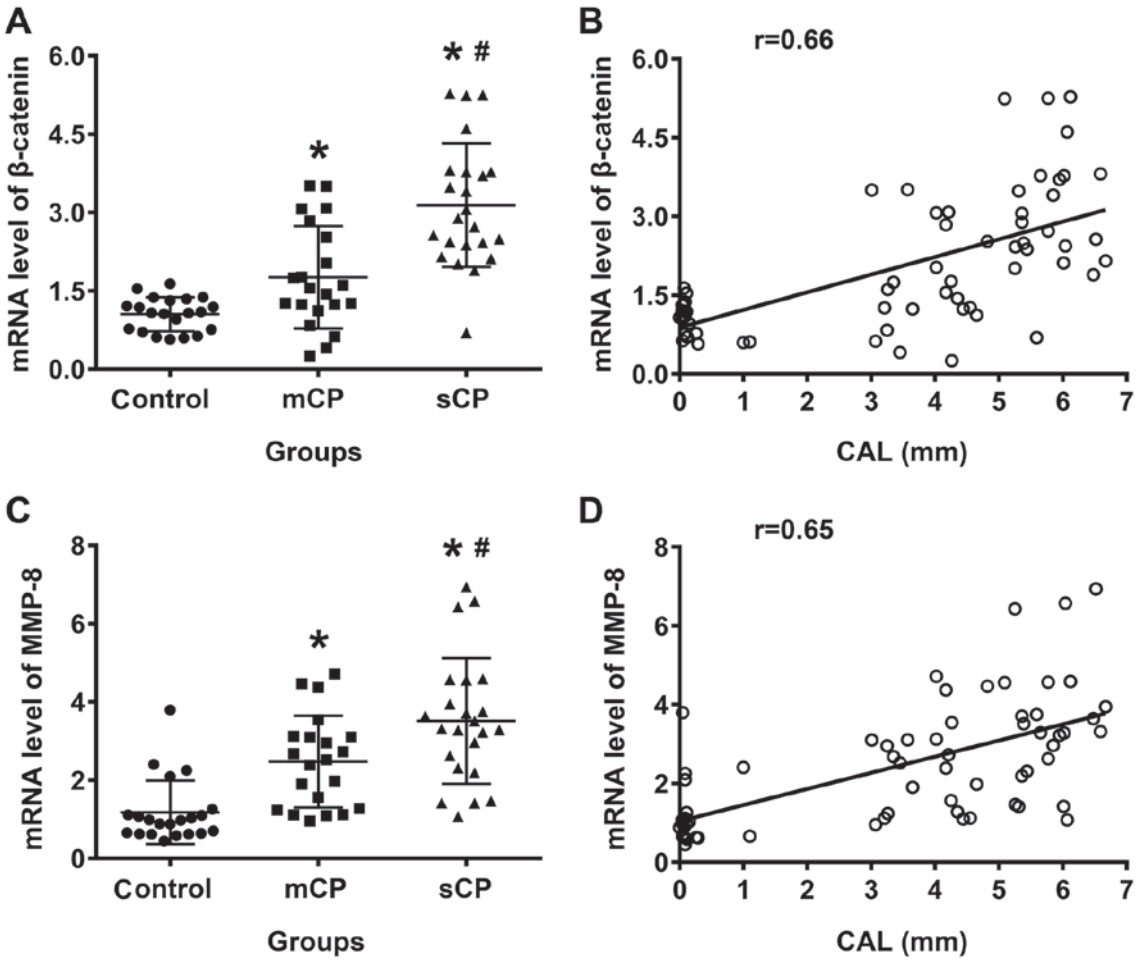

Figure 2. mRNA expression of β-catenin and MMP-8 in GCF. (A) β-catenin mRNA expression in GCF was detected via RT-qPCR. (B) Spearman's rank correlation analysis indicated that the mRNA levels of β-catenin were positively correlated with CAL (r=0.66). (C) mRNA expression of MMP-8 in GCF was detected via RT-qPCR. (D) Spearman's rank correlation analysis indicated that the mRNA expression of MMP-8 was positively correlated with CAL (r=0.65). *P<0.05 vs. the control group; #P<0.05 vs. the mCP group. MMP-8, matrix metalloproteinase; GCF, gingival crevicular fluid; RT-qPCR, reverse transcription-quantitative PCR; CAL, clinical attachment level; mCP, moderate chronic periodontitis; sCP, severe chronic periodontitis.

Statistical significance was determined using one-way ANOVA followed by a post-hoc Tukey's test. χ^2 test was used to determine the statistical significance of sex among groups. A Spearman's rank correlation coefficient was used to determine the association between the protein expression and the clinical periodontal parameters. The analyses and graphs were plotted using GraphPad Prism 6.07 (GraphPad Software Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical parameters. A χ^2 test indicated that there was no significant difference in the sex distributions of the groups (P>0.05; Table I). One-way ANOVA followed by a post-hoc Tukey's test indicated that there was no statistically significant difference in the age of the patients amongst the groups (P>0.05; Table I). The periodontal clinical parameters (PD, CAL, PI and BOP) of the sample sites in the mCP and sCP groups were higher compared with the control group (P<0.05; Table I). Additionally, the PD and CAL of the sample sites in the sCP group were higher compared with the mCP group (P<0.05; Table I). However, the PI and BOP of the sample sites in sCP group were lower compared with the mCP group (P<0.05; Table I).

Concentration of MMP-8 in GCF. The concentration of MMP-8 was detected via ELISA. The concentration of MMP-8 in the mCP and sCP groups was significantly higher compared with the control group (P<0.05; Fig. 1A). Furthermore, the level of MMP-8 in the sCP group was significantly higher compared with the mCP group (P<0.05; Fig. 1A). There was a positive correlation between MMP-8 concentration and CAL in GCF (r=0.79; P<0.05; Fig. 1B).

mRNA expression of β-catenin and MMP-8 in GCF. The mRNA level of β-catenin was significantly higher in patients with chronic periodontitis (both mCP and sCP) compared with control patients (P<0.05; Fig. 2A). Furthermore, the sCP group exhibited a significantly higher β-catenin mRNA expression compared with the mCP group (P<0.05; Fig. 2A). CAL results also revealed a positive correlation with β-catenin mRNA expression (r=0.66; P<0.05; Fig. 2B). The mRNA expression of MMP-8 was significantly higher in patients with chronic periodontitis (both mCP and sCP) compared with the controls (P<0.05; Fig. 2C). Furthermore, the sCP group exhibited a

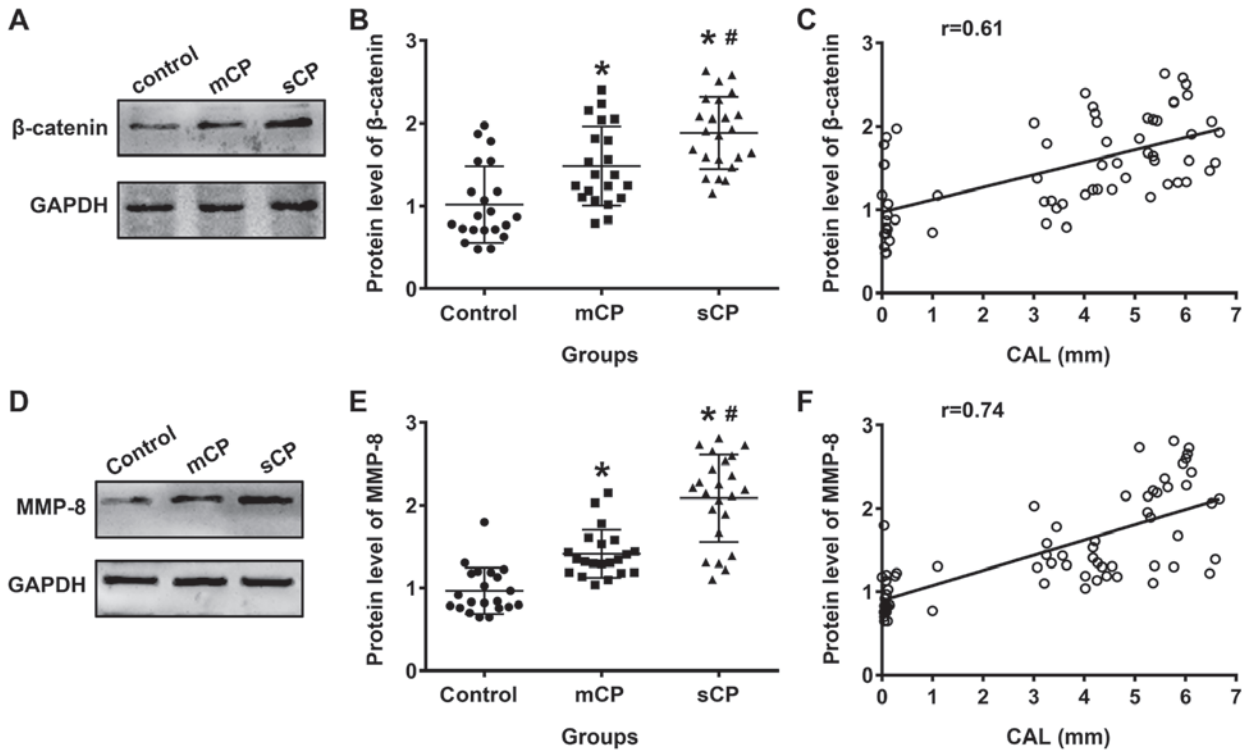

Figure 3. Protein levels of β-catenin and MMP-8 in gingival tissue. (A) Representative western blotting bands of β-catenin. (B) Protein levels of β-catenin in gingival tissue was detected via western blotting, the results of which were normalized to that of GAPDH. (C) Spearman's rank correlation analysis indicated that the protein levels of β-catenin were positively correlated with CAL (r=0.61). (D) Representative western blotting results of MMP-8. (E) Protein levels of MMP-8 in gingival tissue was detected via western blotting, the results of which were normalized to GAPDH. (F) Spearman's rank correlation analysis indicated that the protein expression of MMP-8 was positively correlated with CAL (r=0.74). *P<0.05 vs. the control group; #P<0.05 vs. the mCP group. MMP-8, matrix metalloproteinase; CAL, clinical attachment level; mCP, moderate chronic periodontitis; sCP, severe chronic periodontitis.

significantly higher MMP-8 mRNA expression compared with the mCP group (P<0.05; Fig. 2C). Additionally, CAL results demonstrated a positive correlation with MMP-8 mRNA levels (r=0.65; P<0.05; Fig. 2D).

Protein expression of β-catenin and MMP-8 in gingival tissue. The protein expression of β-catenin was significantly higher in the patients with chronic periodontitis (both mCP and sCP) compared with the controls (P<0.05; Fig. 3A and B) and the sCP group exhibited higher β-catenin protein levels compared with the mCP group (P<0.05; Fig. 3A and B). CAL results revealed a positive correlation with β-catenin protein expression levels (r=0.61; P<0.05; Fig. 3C). The protein levels of MMP-8 were significantly higher in patients with chronic periodontitis (both mCP and sCP) compared with healthy controls (P<0.05; Fig. 3D and E) and the sCP group exhibited higher MMP-8 protein levels compared with the mCP group (P<0.05; Fig. 3D and E). Furthermore, CAL results demonstrated a positive correlation with MMP-8 protein levels (r=0.74; P<0.05; Fig. 3F).

mRNA expression of β-catenin and MMP-8 in gingival tissue. The mRNA expression of β-catenin was significantly higher in patients with chronic periodontitis (both mCP and sCP) compared with the healthy controls (P<0.05; Fig. 4A). Furthermore, the sCP group exhibited higher β-catenin mRNA levels compared with the mCP group (P<0.05; Fig. 4A). A positive correlation was also determined between CAL and β-catenin mRNA expression (r=0.67; P<0.05; Fig. 4B). The mRNA expression of MMP-8 was significantly higher in patients with chronic periodontitis (both mCP and sCP) compared with the controls (P<0.05; Fig. 4C), with the sCP group demonstrating higher MMP-8 mRNA levels compared with the mCP group (P<0.05; Fig. 4C). The results of CAL revealed a positive correlation with MMP-8 mRNA expression (r=0.63; P<0.05; Fig. 4D).

β-catenin interacts with MMP-8 in GCF and gingival tissues. Co-immunoprecipitation was performed to determine whether there was an interaction between β-catenin and MMP-8 in gingival tissue. The results revealed that β-catenin had a positive interaction with MMP-8 in gingival tissue (Fig. 5A). Spearman's rank correlation analysis demonstrated that there was a positive correlation between β-catenin and MMP-8 in mRNA expression levels in GCF (r=0.59; P<0.05; Fig. 5B), and in protein expression levels (r=0.59 and P<0.05; Fig. 5C) and in mRNA expression levels (r=0.61 and P<0.05; Fig. 5D) in gingival tissue.

Discussion

The present study examined the expression of β-catenin and MMP-8 in the GCF and gingival tissues of patients with differing severities of chronic periodontitis. In both GCF and gingival tissue, patients with sCP exhibited higher β-catenin and MMP-8 levels compared with patients with mCP. Additionally, positive interactions between β-catenin and MMP-8 were detected in gingival tissue. These data

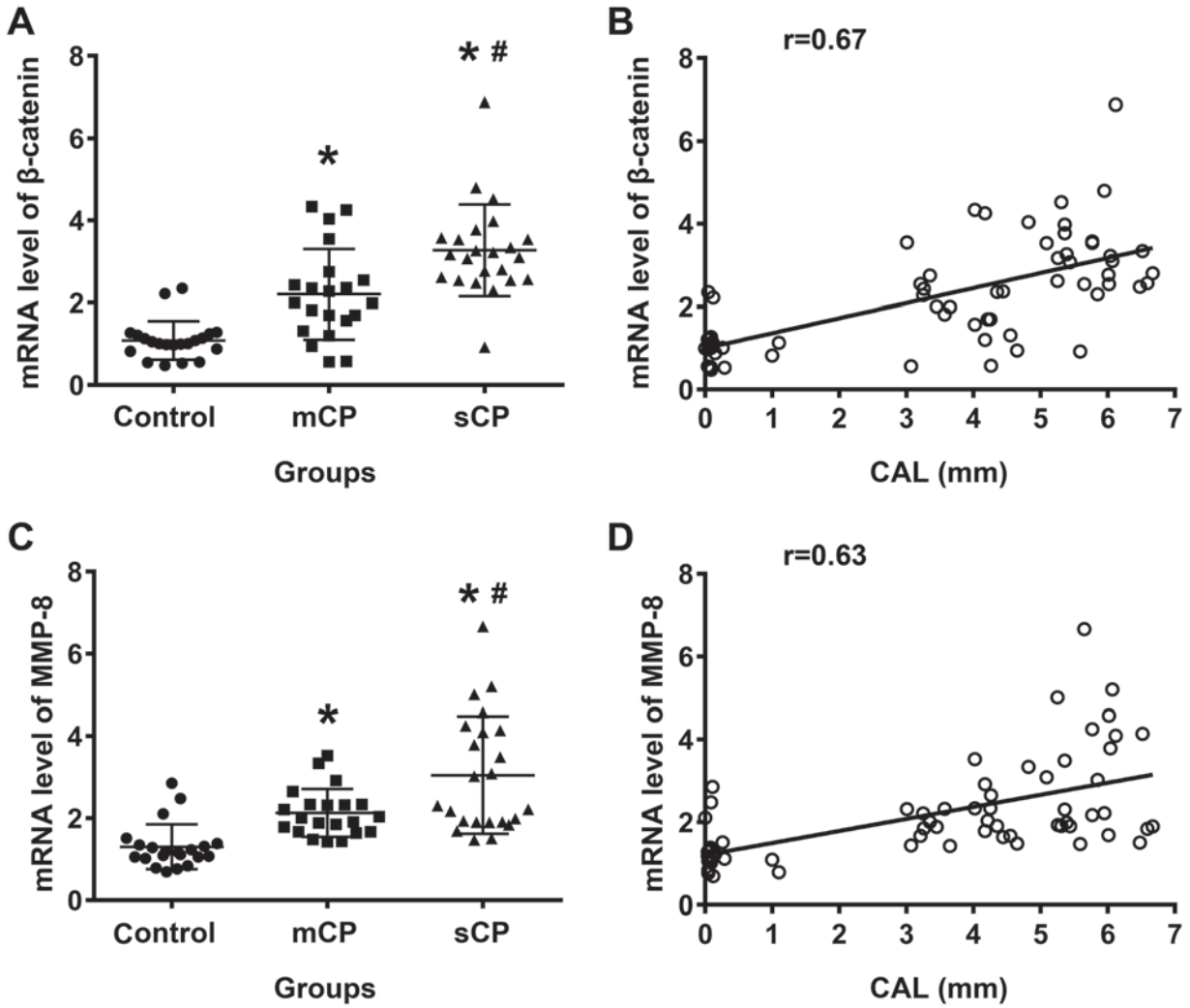

Figure 4. mRNA expression of β-catenin and MMP-8 in gingival tissues. (A) mRNA expression of β-catenin in gingival tissue was detected via RT-qPCR. (B) Spearman's rank correlation analysis demonstrated that there was a positive correlation between the mRNA levels of β-catenin and CAL (r=0.67). (C) mRNA levels of MMP-8 in gingival tissue was detected via RT-qPCR. (D) Spearman's rank correlation analysis demonstrated that the mRNA levels of MMP-8 in gingival tissue was positively correlated with CAL (r=0.63). *P<0.05 vs. the control group; #P<0.05 vs. the mCP group. MMP-8, matrix metalloproteinase; RT-qPCR, reverse transcription-quantitative PCR; CAL, clinical attachment level; mCP, moderate chronic periodontitis; sCP, severe chronic periodontitis.

suggested that the expression of β-catenin and MMP-8 in GCF and gingival tissue may indicate the severity of chronic periodontitis.

Oral fluids (including GCF, gingival crevicular fluid and saliva) have been reported to be rich in serum proteins, inflammatory factors, growth factors, nutrients, microorganisms and metabolites (32). They also provide the microenvironment for the maintenance of oral health and may participate in disease-genesis (32). Therefore, the collection and analysis of oral fluid samples is used as an indicator of oral health and disease (33). In particular, it was previously demonstrated that MMP-8 levels were higher in the saliva of patients with periodontitis compared with healthy controls and MMP-8 in patient saliva may be a crucial biomarker for periodontitis (21,22,34). Similarly, the present study revealed that MMP-8 expression was significantly increased in GCF and gingival tissue. Furthermore, it was also demonstrated that MMP-8 expression was significantly associated with CAL, which is a clinical parameter of periodontitis severity. CAL is often used to classify periodontal diseases and conditions (23). Therefore, elevated levels of MMP-8 in GCF may indicate the severity of chronic periodontitis. Periodontitis is hypothesized to be caused by an interaction between a bacterial infection and the host's immune response, during which MMP-8 has been suggested to be a central mediator in chronic infection-induced inflammatory conditions (1,8,32). Therefore, MMP-8 is implicated in the occurrence and development of periodontitis by regulating collagen degradation and the inflammatory response.

β-catenin is associated with oral diseases and it has a variety of functions dependent on its cellular localization (15). Membrane β-catenin forms a complex with the adhesion molecule, E-cadherin, promoting cell-cell adhesion and contributing to the structural formation of the stratified squamous epithelium of the oral mucosa (35). Cytoplasmic β-catenin is essential for signal transduction from the membrane to the nucleus, where it functions as a transcription factor (36). Nuclear β-catenin as a transcription factor requires

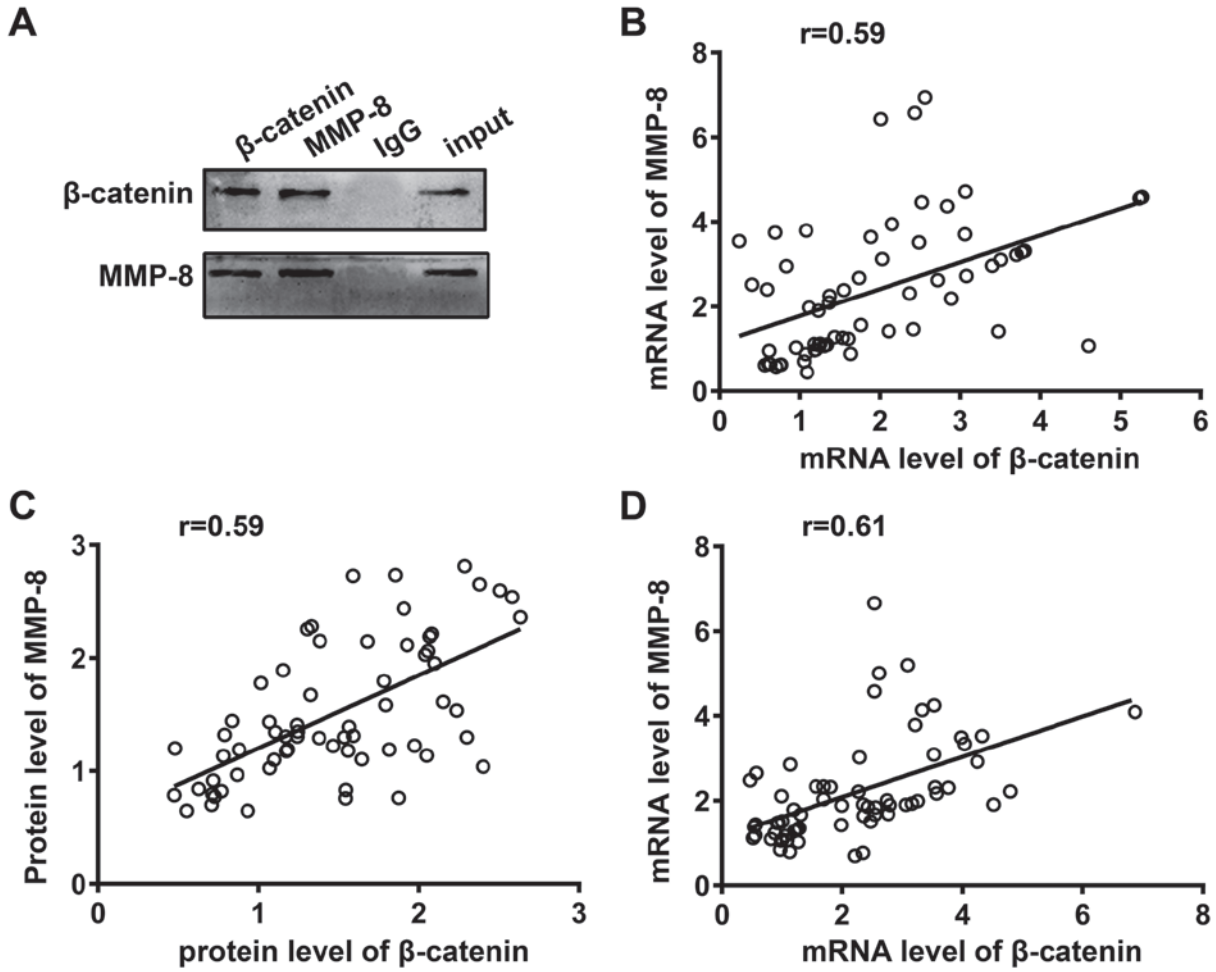

Figure 5. Interaction between β-catenin and MMP-8 in GCF and gingival tissue. (A) Representative western blot of co-immunoprecipitation of β-catenin and MMP-8. (B) Spearman's rank correlation analysis demonstrated that: (B) The mRNA levels of β-catenin in GCF had a positive correlation with the mRNA levels of CGF MMP-8; (C) the protein expression levels of β-catenin in gingival tissue had a positive correlation with the protein expression of gingival tissue MMP-8; and (D) the mRNA levels of β-catenin in gingival tissue had a positive correlation with the mRNA expression of gingival tissue MMP-8. MMP-8, matrix metalloproteinase; GCF, gingival crevicular fluid.

binding to a member of the LEF/TCF family, which exhibit a diverse range of effects on the oral ectoderm, promoting tooth development in certain locations and taste bud formation in others, as well as being required in a host of other critical functions, including skeletal development and lip and palate fusion (35). Napimoga *et al* (37) concluded that expression of dickkopf (a regulator of the Wnt/β-catenin signaling pathway) was increased in the gingival tissue of patients with chronic periodontitis. It was also indicated that it may serve a key regulatory role in determining the outcome of bones in inflammatory environments and in the modulation of the Wnt/β-catenin signaling pathway. Therefore, dickkopf may serve as a potential therapeutic option to prevent bone destruction in endodontic disease (37,38). The present study revealed that the expression of β-catenin was elevated in the GCF and gingival tissue of patients with chronic periodontitis, and that this change was positively correlated with CAL. These results also indicated the possibility of β-catenin serving as a promising therapeutic target for treating patients with chronic periodontitis. Previous studies have suggested that the binding of β-catenin and TCF/LEF may mediate gene transcription, including for those of the ECM (12-15). Brown-Clay *et al* (39) demonstrated that β-catenin-TCF/LEF-mediated MMP production and invasion may be involved in tumorigenesis. However, to the best of our knowledge, there are no studies investigating the association between β-catenin and MMPs in chronic periodontitis. Liu *et al* (11) reported higher levels of β-catenin, MMP-2 and MMP-9 in the gingival tissues of patients with chronic periodontitis compared with controls. The present study revealed a positive interaction between β-catenin and MMP-8 in the gingival tissue of patients with chronic periodontitis, indicating that increased β-catenin may be associated with chronic periodontitis by regulating the expression of MMP-8.

In conclusion, the current study demonstrated that the expression of β-catenin and MMP-8 in GCF and gingival tissue may indicate the severity of chronic periodontitis in patients, thus providing a basis for further study on the possible relevance of β-catenin and MMP-8 as a potential therapeutic target in periodontitis, and to improve our understanding of its pathogenesis. However, there are certain limitations to the present study. It was not possible to detect the time-course change of both β-catenin and MMP-8 levels as the repeated collection of gingival crevicular fluid and gingival tissue from

the same patient was impractical. Additionally, the detailed molecular mechanisms underlying the β-catenin-mediated regulation of MMP-8 expression in chronic periodontitis is remains unknown and requires further study.

Acknowledgements

The authors would like to thank the patients and their families for the donation of GCF and gingival tissues.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed for the present study are available from the corresponding author upon reasonable request.

Authors' contributions

LZ and HX conceived the study, participated in its design and coordination, and drafted the manuscript. YY, JL and JY searched the literature, collected the data, participated in the design of the study and performed statistical analyses. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by The Ethics Committee of Changsha Stomatological Hospital (Changsha, China; approval no. 2015-3).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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