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

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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
the supragingival plaque from interproximal surfaces using a probe (Hu-Friedy Mfg., Co., LLC). The present study (29,30) used a Manual William's periodontal probe (Hu-Friedy Mfg., Co., LLC) for the preceding 6 months. Written informed consent was obtained from all participants or their linear relatives. A total of 21 subjects were included in the 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; mean 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 H₂O₂, 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⁻ΔΔCq method (31). The primer sequences were as follows (all 5'-3'): β-catenin forward, CTCTGAGAAACTTGTCCGATG and reverse, GTGACCACATTTATATCATCAGAAC; MMP-8 forward, AGTTGCCTGACAGTGTTGTT and reverse, TTCCTGTGAGATCCTGGTGA; MMP-8 forward, GCACCGTCAAGGCTGAGAAC and reverse, GGCTGAGACGCCCAGTTGGA.

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
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. ab53017) 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. ab53017) 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. ab53017) 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. ab53017) 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. ab53017) 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. ab53017) 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. ab53017) and rabbit anti-GAPDH (1:4,000; Abcam; cat. no. ab181602).

**Statistical analysis.** Data are expressed as the mean ± standard deviation of at least three independent experiments.
Statistical significance was determined using one-way ANOVA followed by a post-hoc Tukey's test. A $\chi^2$ 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 $\chi^2$ 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 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 (r=0.79; P<0.05; Fig. 1B).

mRNA expression of $\beta$-catenin and MMP-8 in GCF. The mRNA level of $\beta$-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 $\beta$-catenin mRNA expression compared with the mCP group (P<0.05; Fig. 2A). CAL results also revealed a positive correlation with $\beta$-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
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). 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 gingival tissue (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...
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
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.

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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 HX conceived the study, participated in its design and coordination, and drafted the manuscript. 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.

References
1. Papapanou PN and Susin C: Periodontitis epidemiology: Is periodontitis under-recognized, over-diagnosed, or both. Periodontol 2000 75: 45-51, 2017.
2. Page RC: The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontol Res 26: 230-242, 1991.
3. Meimandi M, Talebi Ardakani MR, Esmaeel Nejad A, Yousefnejad P, Saebi K and Tayeef MH: The effect of photodynamic therapy in the treatment of chronic periodontitis: A review of literature. J Lasers Med Sci 8 (Suppl 1): S7-S11, 2017.
4. Manresa C, Sanz-Miralles EC, Twigg J and Bravo M: Supportive periodontal therapy (SPT) for maintaining the dentition in patients with periodontal disease. J Periodontal Res 26: 230-242, 1991.
5. Tjäderhane L, Håkansson I, Reinganum MR, Halme P, Kornman KS, Kojima H, Kajander A, van der Schueren MA, D’Hooghe MT, Astudillo-Diaz M, Marshall JR, Martin RB, Wright GM, Miller FJ, Haffajee AD, Cazalet H, Buser D, Tarnow DP, Eppendorf BC, Socransky SS, Alaluusua S, Egerud G, Dahlen G, Takeshita A, billingham RE, Loesche WJ, Socransky SS: Periodontal therapy (SPT) for maintaining the dentition in patients with periodontal disease. J Periodontal Res 26: 230-242, 1991.
6. Matsui H, Yamasaki M, Nakata K, Amano K and Nakamura H: Expression of MMP-8 and MMP-13 in the development of periodontal lesions. J Endod J 44: 739-745, 2011.
7. Szuba T, Tjäderhane L, Konttinen YT, Lauhos A, Salo T, Lee HM, Golub LM, Brown DL and Mäntylä P: Matrix metalloproteinases: Contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Ann Med 38: 306-321, 2006.
8. Ingman T, Tervahartiala T, Ding Y, Tschesche H, Haerian A, Kinane DF, Konttinen YT and Sorsa T: Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. J Clin Periodontol 23: 1127-1132, 1996.
9. Rathnayake N, Akerman S, Klinge B, Lundegren N, Jansson H, Tresylu S, Yrsa T and Gustafsson A: Salivary biomarkers of oral health: A cross-sectional study. J Clin Periodontol 40: 109-118, 2013.
10. Li X, Zhang Z, Pan S, Shang S and Li C: Interaction between the Wnt/β-catenin signaling pathway and the EMMPRIN/MMP-2, 9 route in periodontitis. J Periodontal Res 53: 842-852, 2018.
11. Hussain M, Xu C, Lu M, Wu X, Tang L and Wu X: Wnt/β-catenin signaling links embryonic lung development and asthmatic airway remodeling. Biochim Biophys Acta Mol Basis Dis 1863: 3226-3242, 2017.
12. Valenta T, Hausmann G and Basler K: The many faces and functions of β-catenin. EMBO J 31: 2714-2736, 2012.
13. Kumawat K, Koopmans T and Gosens R: β-catenin as a regulator and therapeutic target for asthmatic airway remodeling. Expert Opin Ther Targets 18: 1023-1034, 2014.
14. Liu F and Millar SE: Wnt/beta-catenin signaling in oral tissue development and disease. J Dent Res 89: 318-330, 2010.
15. Li YJ, Wei ZM, Meng YX and Ji XR: Beta-catenin up-regulates the expression of cyclinD1, c-myc and MMP-7 in human pancreatic cancer: Relationships with carcinogenesis and metastasis. World J Gastroenterol 11: 2117-2123, 2005.
16. Doyle J and Haas TL: Differential role of beta-catenin in VEGF and histamine-induced MMP-2 production in microvascular endothelial cells. J Cell Biochem 107: 272-283, 2009.
17. Mikeli M, Salo T, Uitto VJ and Larjava H: Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: Cellular origin and relationship to periodontal status. J Dent Res 73: 1397-1406, 1994.
18. Smith PC, Muñoz VC, Collados L and Oyarzún AD: In situ detection of matrix metalloproteinase-9 (MMP-9) in gingival epithelium in human periodontal disease. J Periodontal Res 39: 87-92, 2004.
19. Mauramo M, Ramsay AM, Mauramo E, Buser A, Tervahartiala T, Sorsa T and Waltimo T: Associations of oral fluid MMP-8 with periodontitis in Swiss adult subjects. Oral Dis 24: 449-455, 2018.
20. Gupta N, Gupta ND, Gupta A, Khan S and Bansal N: Role of salivary matrix metalloproteinase-8 (MMP-8) in chronic periodontitis diagnosis. Front Med 9: 72-76, 2015.
21. Konopka L, Pietrzak A and Brzezińska-Błaszczyk E: Effect of scaling and root planing on interleukin-1β, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients. J Periodontal Res 47: 681-688, 2012.
22. Armitage GC: Development of a classification system for periodontal diseases and conditions. Northwest Dent 79: 31-35, 2000.
23. Glavind L and Løe H: Errors in the clinical assessment of periodontal destruction. J Periodontol Res 2: 180-184, 1967.
24. Ainamo J and Bay I: Problems and proposals for recording gingivitis and plaque. Int Dent J 25: 229-235, 1975.
25. Greenstein G: The role of bleeding upon probing in the diagnosis of periodontal disease. A literature review. J Periodontol 55: 684-698, 1984.
26. Offenbacher S, Odle BM and Van Dyke TE: The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. J Periodontal Res 21: 101-112, 1986.
27. Uematsu S, Mogi M and Deguchi T: Interleukin (IL)‑1 beta, IL-6, tumor necrosis factor -alpha, epidermal growth factor, and histamine -induced MMP -2 production in microvascular endothelial cells. J Cell Biochem 107: 272-283, 2009.
28. Kornman KS, Reinganum MR, Halme P, Kajander A, van der Schueren MA, D’Hooghe MT, Astudillo-Diaz M, Marshall JR, Martin RB, Wright GM, Miller FJ, Haffajee AD, Cazalet H, Buser D, Tarnow DP, Eppendorf BC, Socransky SS, Alaluusua S, Egerud G, Dahlen G, Takeshita A, billingham RE, Loesche WJ, Socransky SS: Periodontal therapy (SPT) for maintaining the dentition in patients with periodontal disease. J Periodontal Res 47: 681-688, 2012.
29. Glavind L and Løe H: Errors in the clinical assessment of periodontal destruction. J Periodontol Res 2: 180-184, 1967.
30. Ainamo J and Bay I: Problems and proposals for recording gingivitis and plaque. Int Dent J 25: 229-235, 1975.
31. Greenstein G: The role of bleeding upon probing in the diagnosis of periodontal disease. A literature review. J Periodontol 55: 684-698, 1984.
32. Offenbacher S, Odle BM and Van Dyke TE: The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. J Periodontal Res 21: 101-112, 1986.
33. Uematsu S, Mogi M and Deguchi T: Interleukin (IL)‑1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. J Dent Res 75: 562-567, 1996.
34. Xie P, Deng LX, Gong P, Ding Y and Tang XH: Expression of HMGB1 and HMGB2 in gingival tissues, GCF and PICP of periodontitis patients and peri-implantitis. Braz J Microbiol 42: 1213-1219, 2011.
35. Petković AB, Matić SM, Stamatović NV, Vojvodić DV, Todorović TM, Lazić ZR and Kozomara RJ: Proinflammatory cytokines (IL-1beta and TNFα-alpha) and chemokines (IL-8 and MIP-1alpha) as markers of peri-implant tissue condition. J Oral Maxillofac Surg 39: 478-485, 2010.
31. Schmittgen TD and Livak KJ: Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3: 1101-1108, 2008.
32. Kinney JS, Ramseier CA and Giannobile WV: Oral fluid-based biomarkers of alveolar bone loss in periodontitis. Ann N Y Acad Sci 1098: 230-251, 2007.
33. Sorsa T, Tervahartiala T, Leppilahdi J, Hernandez M, Gamonal J, Tuomainen AM, Lauhio A, Pussinen PJ and Mäntylä P: Collagenase-2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. Pharmacol Res 63: 108-113, 2011.
34. Zhang L, Li X, Yan H and Huang L: Salivary matrix metalloproteinase (MMP)-8 as a biomarker for periodontitis: A PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore) 97: e9642, 2018.
35. González-Moles MA, Ruiz-Ávila I, Gil-Montoya JA, Plaza-Camplillo J and Scully C: β-catenin in oral cancer: An update on current knowledge. Oral Oncol 50: 818-824, 2014.
36. MacDonald BT, Tamai K and He X: Wnt/beta-catenin signaling: Components, mechanisms, and diseases. Dev Cell 17: 9-26, 2009.
37. Napimoga MH, Nametala C, da Silva FL, Miranda TS, Bossonaro JP, Demasi AP and Duarte PM: Involvement of the Wnt-β-catenin signalling antagonists, sclerostin and dickkopf-related protein 1, in chronic periodontitis. J Clin Periodontol 41: 550-557, 2014.
38. Tan X, Huang D, Zhou W, Yan L, Yue J, Lu W, Song D, Zhou X, Ye L and Zhang L: Dickkopf-1 may regulate bone coupling by attenuating wnt/β-catenin signaling in chronic apical periodontitis. Arch Oral Biol 86: 94-100, 2018.
39. Brown-Clay JD, Shenoy DN, Timofeeva O, Kallakury BV, Nandi AK and Banerjee PP: PBK/TOPK enhances aggressive phenotype in prostate cancer via β-catenin-TCF/LEF-mediated matrix metalloproteinases production and invasion. Oncotarget 6: 15594-15609, 2015.

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