


RESEARCH ARTICLE

Treponema denticola increases MMP-2 expression and activation in the periodontium via reversible DNA and histone modifications

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Abstract

Host-derived matrix metalloproteinases (MMPs) and bacterial proteases mediate destruction of extracellular matrices and supporting alveolar bone in periodontitis. The *Treponema denticola* dentilisin protease induces MMP-2 expression and activation in periodontal ligament (PDL) cells, and dentilisin-mediated activation of pro-MMP-2 is required for cellular fibronectin degradation. Here, we report that *T. denticola* regulates MMP-2 expression through epigenetic modifications in the periodontium. PDL cells were treated with epigenetic enzyme inhibitors before or after *T. denticola* challenge. Fibronectin fragmentation, MMP-2 expression, and activation were assessed by immunoblot, zymography, and qRT-PCR, respectively. Chromatin modification enzyme expression in *T. denticola*-challenged PDL cells and periodontal tissues were evaluated using gene arrays. Several classes of epigenetic enzymes showed significant alterations in transcription in diseased tissue and *T. denticola*-challenged PDL cells. *T. denticola*-mediated MMP-2 expression and activation were significantly reduced in PDL cells treated with inhibitors of aurora kinases and histone deacetylases. In contrast, DNA methyltransferase inhibitors had little effect, and inhibitors of histone acetyltransferases, methyltransferases, and demethylases exacerbated *T. denticola*-mediated MMP-2 expression and activation. Chronic epigenetic changes in periodontal tissues mediated by *T. denticola* or other oral microbes may contribute to the limited success of conventional treatment of chronic periodontitis and may be amenable to therapeutic reversal.

1 | INTRODUCTION

Periodontitis, a bacterially mediated chronic inflammatory disease of the tissues supporting the tooth is one of the most common inflammatory diseases in humans, and it can adversely affect systemic health (Armitage, 2004; Armitage, 2008). National surveys show that the majority of adults suffer from mild-to-moderate periodontitis, with up to 15% of the population being affected by severe forms at some stage in their lives (Pihlstrom, Michalowicz, & Johnson, 2005). Periodontitis causes largely irreversible destruction of the periodontal tissues, and in advanced stages, tooth loss,

speech and masticatory problems, and an overall reduced quality of life. Moreover, the systemic burden of periodontitis on different organs has been investigated. It has been reported that periodontal infections can adversely predispose to coronary heart diseases (Stewart & West, 2016), ischaemic strokes (Leira et al., 2016), poor glycaemic control (Garcia, Tarima, & Okunseri, 2015), preterm labour, low-birth-weight delivery (Sitholimela & Shangase, 2013), and pulmonary diseases (Prasanna, 2011). These systemic effects have been attributed to either direct bacterial invasion or modulation of specific host inflammatory and tissue destructive mediators (Williams et al., 2008).

Destruction of the periodontal extracellular matrices (ECM) and detrimental changes in the cellular elements of the periodontal ligament (PDL) occur as a result of disruption of normal tissue homeostatic processes. One of these disrupted processes is regulation of host-derived matrix metalloproteinases (MMPs) that are both directly and indirectly involved in periodontal tissue breakdown. Destruction of the ECM in periodontitis results in the release of fibronectin (FN) fragments, which are considered markers of periodontal disease status (Huynh et al., 2002). Evidence from in vitro studies further indicate that these FN fragments induce several detrimental effects, including induction of apoptosis and suppression of osteoblast differentiation of PDL cells (Dai, Iwama, Wang, & Kapila, 2005; Ghosh, Park, Fenno, & Kapila, 2008; Jee, Wang, & Kapila, 2004; Joseph, Kapila, Hayami, & Kapila, 2010; Kapila, Kapila, & Johnson, 1996; Kapila, Lancero, & Johnson, 1998; Kapila, Wang, & Johnson, 1999), thereby further potentiating disease progression.

MMPs are synthesised in latent form and then activated through proteolytic cleavage to expose the catalytic site of the MMP enzyme. MMP activation is primarily extracellular, though intracellular activation is reported in certain cases (Murphy et al., 1999; Nagase, 1997). MMPs are synthesised at low basal levels for maintenance of homeostatic processes; however, their levels and activation are typically increased during disease (Mittal et al., 2016). In addition to their role in remodelling the ECM and basement membrane during various physiologic processes, MMPs are implicated in a wide range of pathologic processes, including cardiovascular (Azevedo, Prado, Antonio, Issa, & Gerlach, 2014), pulmonary (Navratilova, Kolek, & Petrek, 2016; Pardo, Cabrera, Maldonado, & Selman, 2016), renal (Aresu, Benali, Garbisa, Gallo, & Castagnaro, 2011; Charitaki et al., 2016), and gastrointestinal diseases (Medina, Santana, Quintero, Radomski, & Guarnier, 2004), cancer (Endres et al., 2016, Ligi & Mannello, 2016, Liu et al., 2016, Lukaszewicz-Zajac, Szmitkowski, Litman-Zawadzka, & Mroczko, 2016, Pietruszewska, Bojanowska-Pozniak, & Kobos, 2016), and inflammatory diseases, such as periodontitis (Nissinen & Kahari, 2014; Shinkarenko, Rumiantsev, Egorova, & Eliseeva, 2013).

MMP-2, one of several MMPs involved in tissue homeostasis and remodelling, is constitutively expressed by PDL cells as pro-MMP-2 (Kapila et al., 1996). Thus, control of the process or rate of activation of MMP-2 has been proposed as a key regulatory step in periodontal tissue homeostasis (Borkham-Kamphorst, Alexi, Tihaa, Haas, & Weiskirchen, 2015; Madsen et al., 2013; Mosig & Martignetti, 2013). Activation of pro-MMP-2 is promoted by proteolytic cleavage by the membrane type-1-MMP (MT1-MMP/MMP-14; Murphy et al., 1999; Nagase, 1997; Zucker, Pei, Cao, & Lopez-Otin, 2003). Extracellular MMP activities are controlled by the blockage of autolytic MMP activation or by endogenous proteinase inhibitors, such as tissue inhibitors of MMPs (TIMPs; Brew & Nagase, 2010; Nagase, Visse, & Murphy, 2006). Regulation of the proteolytic activity of MMP-2 is dependent on the balance between MT1-MMP/TIMP-2 (Hernandez-Barrantes, Shimura, Soloway, Sang, & Fridman, 2001; Oyarzun et al., 2010; Shofuda et al., 1998). This mechanism of MMP-2 activation by an MT1-MMP-TIMP-2 complex has been well recognised in other systems (Butler et al., 1998; Kinoshita et al., 1998; Strongin et al., 1995). Disturbances in that balance

may result in excessive tissue degradation associated with inflammatory diseases (Ejeil et al., 2003).

Treponema denticola along with *Porphyromonas gingivalis* and *Tannerella forsythia* becomes prevalent in late stages of subgingival biofilm formation and comprises the bacterial "red complex" that is considered pathogenic in the aetiology of periodontal disease (Socransky, Haffajee, Cugini, Smith, & Kent Jr., 1998). Although our understanding of the periodontal disease microbiome has greatly increased, the "red complex" bacteria continue to be recognised as important pathogens in the disease process. Oral spirochetes including *T. denticola* often predominate in periodontal disease, though they are typically below detectable levels in healthy gingival plaque (Choi, Paster, Dewhirst, & Gobel, 1994; Ellen & Galimanas, 2005). The levels of *T. denticola* increase with the severity of periodontitis, underscoring its major role in the disease (Simonson, Goodman, Bial, & Morton, 1988; Yoshida et al., 2004).

Recognised virulence factors of *T. denticola* include the acylated serine protease complex (dentilisin; PrtP complex; CTLP/chymotrypsin-like protease) that degrades gelatin, laminin, and various serum components and bioactive peptides (Grenier, Uitto, & McBride, 1990; Makinen, Makinen, & Syed, 1995; Uitto, Grenier, Chan, & McBride, 1988). The dentilisin complex contributes to *T. denticola* adherence and cytotoxic effects on epithelial cells and fibroblasts (Ellen, Dawson, & Yang, 1994; Fenno et al., 1998; Mathers, Leung, Fenno, Hong, & McBride, 1996) and penetration of epithelial tissue (Chi, Qi, & Kuramitsu, 2003), and it may play a role in complement-mediated bactericidal activity (McDowell, Huang, Fenno, & Marconi, 2009) and complement evasion (McDowell et al., 2011). Of particular relevance to the current study, we previously demonstrated that dentilisin proteolytic activity induces activation of pro-MMP-2 in cultured PDL cells and that activated MMP-2 is required for cleavage of cellular FN into fragments similar to those observed in gingival crevicular fluid from periodontal lesions (Miao, Fenno, Timm, Joo, & Kapila, 2011). Furthermore, transcription and expression of MT1/MMP and TIMP-2 increased in response to *T. denticola* challenge (Miao et al., 2014). Taken together, these properties suggest important links between the *T. denticola* protease activity and regulation of the cellular and tissue processes that result in periodontal tissue destruction.

Epigenetics is defined as heritable and potentially reversible changes in gene expression without alterations in the DNA sequence (Goldberg, Allis, & Bernstein, 2007; Waddington, 2012). Such modifications are not only associated with diseases but are also essential for the incorporation and integration of endogenous and environmental signals in cells. Epigenetic status can be affected by environmental factors, such as nutrients, toxins, infections, and hypoxia, with subsequent upregulation or downregulation of specific gene expression patterns (Barros & Offenbacher, 2009; Bayarsaihan, 2011, Safronova & Morita, 2010, Yin & Chung, 2011). Emerging evidence suggests that epigenetic modifications play a major role in inflammatory diseases, including periodontal disease (Barros & Offenbacher, 2014). Several factors that mediate periodontal disease pathogenesis, including bacteria and their by-products, smoking, and diabetes, induce marked epigenetic changes in tissue components (Khansari, Shakiba, & Mahmoudi, 2009; Martinez, Milagro, Claycombe, & Schalinske, 2014; Medzhitov & Horng, 2009; Offenbacher, Barros, &

Beck, 2008; Pasquier, Hoarau-Vechot, Fakhro, Rafii, & Abi Khalil, 2015; Razzouk & Sarkis, 2013; Yin & Chung, 2011). For example, chronically inflamed periodontal tissues demonstrated an increased methylation of CpG-rich regions of the PTGS2/COX2 promoter compared to healthy periodontal tissues (Zhang et al., 2010). Also, there is marked hypomethylation of the IL8 promoter in oral epithelial cells of subjects with generalised aggressive periodontitis compared to control subjects (Andia et al., 2010). Emerging studies suggest that microbial pathogens, including oral species such as *P. gingivalis*, induce epigenetic modifications in host cells (reviewed in Niller, Masa, Venkei, Meszaros, & Minarovits, 2017). We recently identified potential epigenetic links between *T. denticola* and genes in PDL cells involved in activation of MMP-2 (Miao et al., 2014). Thus, *T. denticola* may mediate epigenetic modifications that regulate MMP-2 activation and subsequent ECM degradation in the periodontium.

Epigenetic modifications are potentially reversible, and therefore, a thorough understanding of these changes may identify new therapeutic targets for disease management. The aim of this study was to investigate *T. denticola*'s ability to chronically activate MMP expression through epigenetic modifications in PDL cells/tissues and to examine potential therapeutic approaches for reversal/modification of these changes.

2 | RESULTS

2.1 | *T. denticola* chronically upregulates expression of MMP-2, MT1-MMP, and TIMP-2, with concomitant FN fragmentation

To determine the long-term effects of a brief exposure to *T. denticola* on MMP-2 expression in host cells, PDL cells were briefly challenged with *T. denticola*, and then MMP-2 expression and MMP-2 activation in long-term cultures with daily medium changes were assessed by gelatin zymography and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). As shown in Figure 1a, PDL cells constitutively expressed basal levels of pro-MMP-2 with minimal activation for maintenance of homeostatic functions. However, challenge with *T. denticola* triggered both chronic increased MMP-2 expression (pro-MMP2) and activation (active MMP-2) in PDL cells. Following a 2-hr exposure to *T. denticola*, dentilisin activity (visible as a ~100 kDa band on the zymogram, Figure 1a) persisted within these cultures throughout the experiment, though it was detected at greatly reduced levels at Days 9 and 12. Expression and activation levels of MMP-2 were chronically sustained for up to 12 days, with minor reductions in the levels of activated MMP-2 on Days 9 and 12 (Figure 1a). *T. denticola*-mediated increases in MMP-2 expression and activation were mirrored by concomitant FN fragmentation throughout the experiment (Figure 1b).

The chronic effects of *T. denticola* on MMP-2 expression in PDL cells were regulated at the transcriptional level. MMP-2 mRNA levels were upregulated for up to 12 days as assessed by qRT-PCR (Figure 1c). Given that the MT1-MMP/TIMP-2 complex is a well-known regulator of MMP-2 activation, MT1-MMP and TIMP-2 expression were examined in *T. denticola* challenged PDL cells in

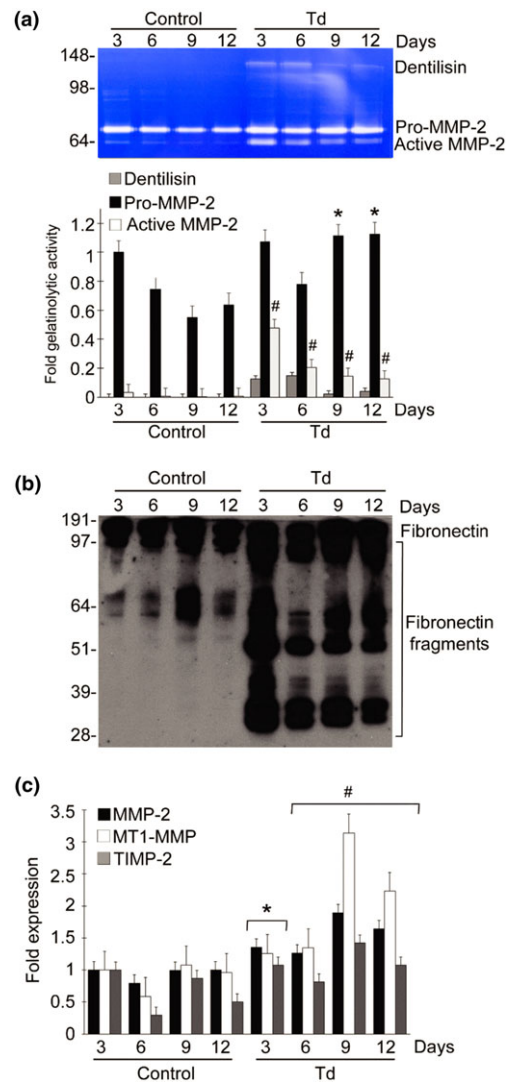


FIGURE 1 *Treponema denticola* mediates chronic expression and activation of MMP-2, MT1-MMP, and TIMP-2 in periodontal ligament (PDL) cells, with subsequent fragmentation of cellular fibronectin. Cultured PDL cells were challenged with *T. denticola* (*Td*) at multiplicity of infection = 100 or media control for 2 hr and then incubated for 3, 6, 9, and 12 days with daily medium changes. The experiments were repeated three times in triplicate. Data were analysed using one-way analysis of variance. (*) represents $p \leq .05$ compared to the same time point in the control group. (#) represents $p \leq .001$ compared to the same time point in the control group. (a) A representative gelatin zymogram of PDL cell conditioned medium showing the gelatinolytic activity of pro-MMP-2 (72-kDa), active MMP-2 (64-kDa), and *Td* dentilisin (100 kDa). The left four lanes represent the control unchallenged PDL cells, and the right four lanes represent the *Td*-challenged PDL cells. The bar chart below represents the densitometric analysis of gelatinolytic activity in the zymograms using ImageJ™-NIH software. The y axis represents fold-gelatinolytic activity of the pro-MMP-2, active MMP-2, and dentilisin relative to unchallenged control at Day 3. The x axis represents different time points. (b) A representative immunoblot of PDL cell culture supernatants probed with a polyclonal anti-fibronectin antibody showing fibronectin fragmentation in conditioned medium from *Td*-challenged PDL cells. (c) Transcript levels of MMP-2, MT1-MMP, and TIMP-2 in PDL cells at different time points after challenge with *Td* or media control assayed by qRT-PCR. The y axis represents fold-expression level of each gene relative to unchallenged control at Day 3. The x axis represents different time points

long-term cultures. Expression of the MT1-MMP/TIMP-2 complex was also chronically upregulated by the *T. denticola* challenge, mirroring the changes induced in MMP-2 transcriptional expression (Figure 1c).

2.2 | *T. denticola* levels and MMP-2 transcription are elevated in periodontal disease

Examination of human tissues from periodontally diseased and healthy sites confirmed the association between *T. denticola* and elevated MMP-2 expression in diseased tissues. Human tissue specimens from periodontally diseased sites exhibited elevated levels of *T. denticola* concomitant with elevated levels of MMP-2 mRNA expression compared with healthy sites (Figure 2). Tissue specimens from healthy sites exhibited negligible levels of *T. denticola* and low levels of MMP-2 expression. Low levels of MMP-2 expression in healthy tissues are consistent with the low basal levels of MMP-2 expression necessary for homeostatic functions within the periodontium, whereas increased MMP-2 expression is consistent with dysbiotic alterations of periodontal homeostasis in disease.

2.3 | Expression of genes encoding chromatin modification enzymes are significantly altered in periodontal disease

To further examine the role of epigenetics in periodontal disease, the major chromatin modification enzymes that might be involved in the disease process (Table S1) were assessed via a focused gene array. Applying a "twofold change" as a threshold value, we found that several chromatin modification enzymes were significantly altered in diseased periodontal tissue specimens compared to healthy control tissues (Figure 3a,b and Table S2). The most significantly altered

enzymes were the histone methyltransferases (HMTs) and histone deacetylases (HDACs), including PRMT8 and HDAC11, which exhibited a significant downregulation. Other enzymes, especially those related to SET domain proteins, histone acetyltransferases (HATs), and histone phosphorylation kinases showed significant upregulation, such as SETD4, ESCO1, ESCO2, and AURKB. Other significantly altered enzymes included HATs, HDACs, histone ubiquitinases, DNA demethylases, and histone demethylases (HDMs), and DNA methyltransferases (DNMTs).

2.4 | *T. denticola* significantly alters expression of genes encoding chromatin modification enzymes in PDL cells

To examine the potential role of epigenetics in the *T. denticola*-mediated upregulation of MMP-2 expression, a focused gene array was employed to study all major epigenetic chromatin modification enzymes that might be involved in this process (Table S1). Applying a twofold change as a threshold value, we found that *T. denticola*-challenged PDL cells exhibited significantly decreased levels of all major chromatin modification enzymes (and Figure 3c,d and Table S3). No chromatin modifying enzymes assayed showed increased expression (data not shown). The most significantly downregulated enzymes included aurora kinases, which mediate histone phosphorylation, and HMTs. Specifically, within these classes of enzymes, the most downregulated enzymes included Aurora Kinase B and EHMT2. Other significantly downregulated enzymes included HATs, HDACs, histone ubiquitinases, DNA demethylases and HDMs, and DNMTs.

2.5 | Inhibitors of histone kinase/aurora kinase, DNMT, and HDAC block *T. denticola*-mediated increases in MMP-2 activity and expression in PDL cells

Given the broad landscape of epigenetic changes mediated by *T. denticola* on PDL cells and the changes exhibited in diseased tissues, targeted approaches were employed to examine the role of representative members of each major class of epigenetic enzymes (Table 1) in the process of MMP-2 modulation and FN fragmentation in *T. denticola*-challenged PDL cells.

Pretreatment of PDL cells with the histone kinase/phosphorylation inhibitor, PF-03814735, inhibited the potential increase in MMP-2 activation and expression mediated by *T. denticola* (Figure 4 a–c). Pretreatment with PF-03814735 also prevented the transcriptional increase in the MMP-2 activator complex, MT1-MMP/TIMP-2, and FN fragmentation mediated by the *T. denticola* challenge. This aurora kinase inhibitor exhibited a dose–response effect in terms of preventing the changes in MMP-2 expression and activation. At the highest dose tested, PF-03814735 also suppressed MMP-2 expression and activation in control cells. All enzyme inhibitor concentrations were selected in ranges that did not alter proliferation or induce cytotoxicity in PDL cells (data not shown).

Prevention of the potential increase in MMP-2 activation and expression mediated by *T. denticola* was also achieved by pretreatment of PDL cells with azacytidine (AZA), a DNMT inhibitor (Figure 5a–c).

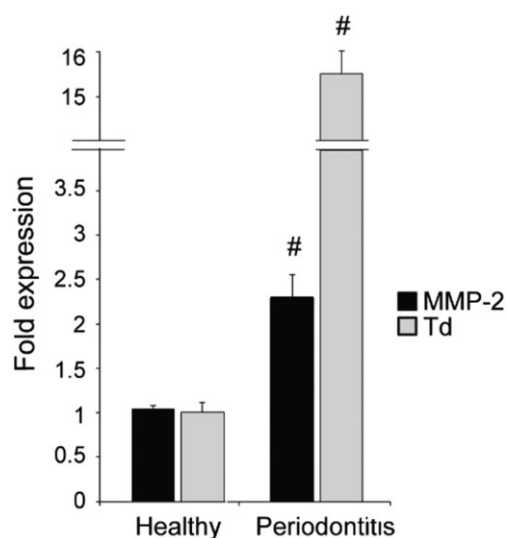


FIGURE 2 Elevated MMP-2 mRNA and *Treponema denticola* in diseased periodontal tissue. Levels of *T. denticola* 16S rRNA and MMP-2 mRNA in tissue specimens from periodontally diseased or healthy sites were assayed by qRT-PCR. The y axis represents levels of each RNA in periodontitis specimens relative to healthy specimens. The x axis represents the source of tissue specimens. Data were analysed using Student's *t* test. (#) represents $p \leq .001$ compared to healthy tissue

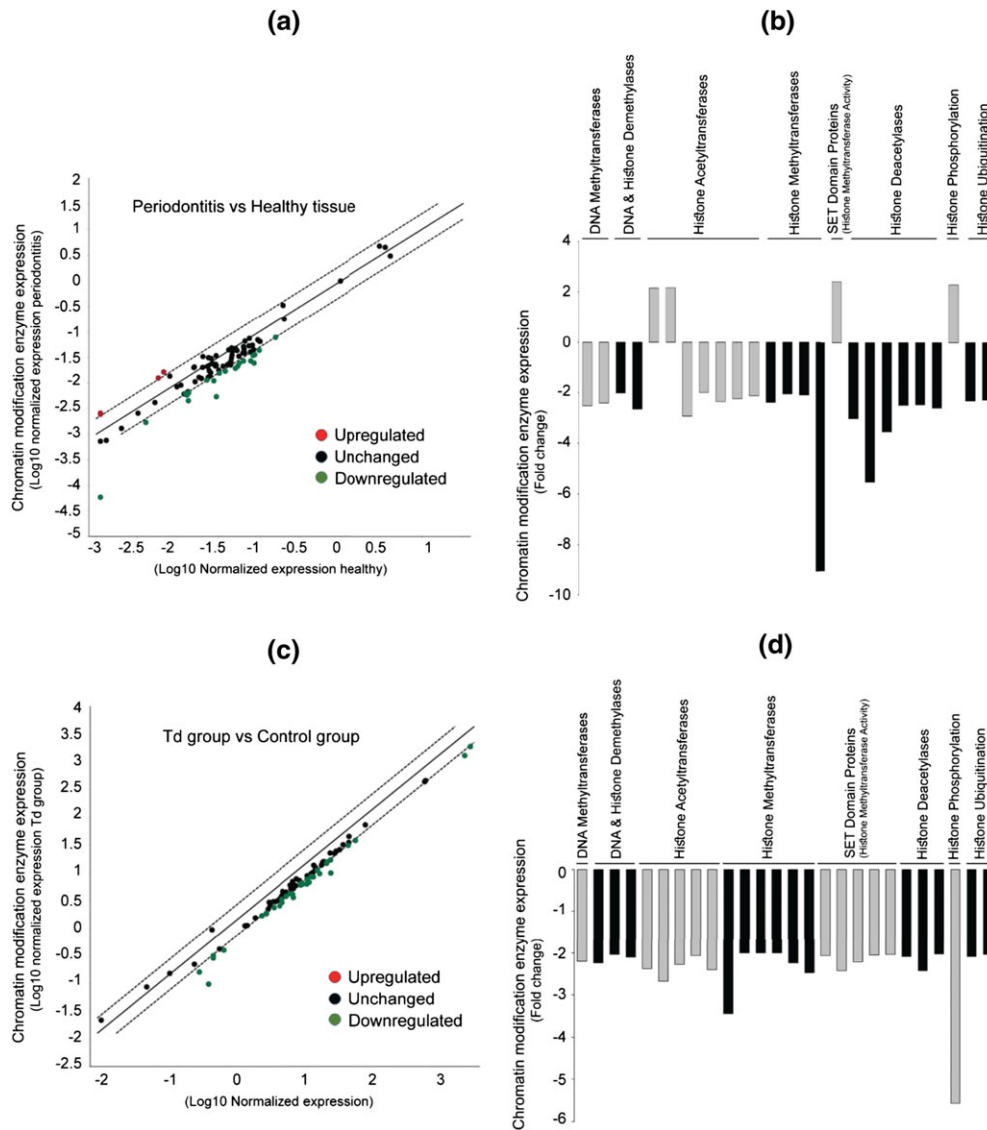


FIGURE 3 Transcription of multiple chromatin modification enzymes is altered in both periodontally diseased tissue and *Treponema denticola*-challenged periodontal ligament (PDL) cells. Transcription levels of 87 chromatin modification enzymes were examined by qRT-PCR using RNA extracted from healthy and diseased periodontal tissues (Panels (a) and (b), respectively) and from PDL cell lysates collected 9 days after *Td* challenge as described in Figure 1 (Panels (c) and (d), respectively). (a and c) Scatter blots showing the transcriptional level of chromatin modification enzymes in periodontally diseased tissues relative to healthy tissue controls (Panel (a)) and in *Td*-challenged PDL cells relative to unchallenged control cells (Panel (c)). The y axis represents expression level of different enzymes relative to healthy tissue (Panel (a)) or unchallenged control cells (Panel (c)). The x axis represents normalised expression of the respective control group. (b and d) Bar charts showing the significantly downregulated chromatin modification enzymes in periodontally diseased tissues relative to healthy tissue controls (Panel (b)) and in *Td*-challenged PDL cells relative to unchallenged control cells (Panel (d)). The y axis represents the fold-expression/downregulation level of enzymes relative to unchallenged controls. The x axis represents different chromatin modification enzymes, with enzyme type grouped by colour

AZA had a dose-response effect, inhibiting increases in MMP-2 expression and activation, MT1-MMP/TIMP-2 expression, and FN fragmentation mediated by the *T. denticola* challenge. In contrast, epigallocatechin gallate (EGCG), a different DNMT inhibitor with a broad spectrum of activity that includes inhibition of HATs, had considerably less effect than AZA. At the highest concentration tested, EGCG had only minor effects on *T. denticola*-mediated MMP-2 activation and TIMP-2 expression, but this did not result in modulation of FN fragmentation (Figure 6a–c).

Examination of HDAC inhibitors apicidin and trichostatin revealed that pretreatment with these two inhibitors at the highest doses tested (1 μ M and 0.1 μ M, respectively) also inhibited the *T. denticola*-

mediated increase in MMP-2 expression and activation as well as that of its activator complex, MT1-MMP/TIMP-2. However, as with EGCG, neither of these inhibitors affected FN fragmentation (data not shown).

2.6 | HMT, HDM, and HAT inhibitors exacerbate *T. denticola*-mediated increases in MMP-2 expression and activity in PDL cells

Inhibitors of HDMs, HMTs, and HATs were not effective in preventing the *T. denticola*-mediated increases in pro-MMP-2 expression and activation. Treatment with curcumin, a HAT inhibitor, resulted in moderately increased expression of pro-MMP-2, but significantly

TABLE 1 Chemical agents used to target chromatin modification enzymes

Compound	Target enzyme	Concentrations (μM)		Treatment period	Source	References
		Low	High			
Inhibition of DNA methyltransferases (DNMT)						
5-Azacytidine	-DNMT	1	5	4 days	^a	(Chernov, Sounni, Remacle, & Strongin, 2009, Hassler et al., 2012, Zhang et al., 2011)
(-)-Epigallocatechin gallate (EGCG)	-DNMT -HAT: P300, CBP, PCAF, TIP60	5	10	2 days	^a	(Achour et al., 2013, Saldanha, Kala, & Tollefsbol, 2014)
Inhibition of histone deacetylases (HDACs)						
Trichostatin A	-HDAC	0.05	0.1	4 days	^a	(Chang et al., 2012, Mogal & Abdulkadir, 2006)
Apicidin	-HDAC	0.5	1	1 day	^a	(Ahn et al., 2012, Bauden, Tassidis, & Ansari, 2015)
Inhibition of histone phosphorylation						
PF-03814735	-AURK A -AURK B	0.25	0.5	2 days	^a	(Hook et al., 2012)
Inhibition of histone acetyltransferases (HATs)						
Curcumin	-HATs	25	50	2 hr	^a	(Ahn et al., 2012, Balasubramanyam et al., 2004)
Inhibition of histone methyltransferases (HMTs)						
BIX 01294	-HMT G9a	0.5	1	2 days	^a	(Kubicek et al., 2007)
Inhibition of histone demethylases (HDMs)						
Tranylcypromine, HCl (TCP)	-Monoamine oxidase A/B Histone LSD1/2	2	5	4 days	^b	(Nebbioso et al., 2012)

^aSigma-Aldrich, St. Louis, MO, USA.

^bEMD Millipore, Temecula, CA, USA.

decreased its MMP-2 activation in a dose-dependent manner (Figure 7 a). In contrast, the HDM inhibitor, tranylcypromine, HCl (TCP) and the HMT inhibitor, BIX 01295/trihydrochloride hydrate further augmented the *T. denticola*-mediated increases in MMP-2 expression and activation in PDL cells (Figure 7b,c).

2.7 | Inhibitors of histone phosphorylation and HDAC reverse *T. denticola*-mediated increases in MMP-2 activity and expression in PDL cells

An important remaining question was whether existing epigenetic modifications on PDL cells mediated by *T. denticola* could be reversed in a post-treatment scenario. To this end, PDL cells were first challenged with *T. denticola* and then the same enzyme inhibitors tested above were employed to address this question. In agreement with the pretreatment results, post-treatment of *T. denticola* challenged PDL cells with three different epigenetic enzyme inhibitors reversed the effects on MMP-2 expression and activation: the histone kinase inhibitor, PF-03814735 (Figure 8a), and the HDAC inhibitors apicidin (Figure 8b) and trichostatin (Figure 8c).

It is important to note that the results of the prechallenge and postchallenge inhibition experiments should not be compared in parallel because of different sample collection times used. In the pretreatment experiments, samples were collected after 3 days of a *T. denticola* (2-hr exposure) challenge, whereas in the postchallenge treatment experiments, the samples were collected 2 hr to 4 days after a *T. denticola* (2 hr exposure) challenge, depending on the inhibitor used. Similarly, in contrast to the prechallenge enzyme inhibition

experiments, no decrease in FN fragmentation was observed in postchallenge inhibition experiments (data not shown), presumably because the MMP-2-mediated fragmentation process had begun before addition of enzyme inhibitors.

3 | DISCUSSION

ECM destruction, a key event in the pathogenesis of periodontitis, is mediated by host-derived enzymes such as MMP-2 that are involved in ECM homeostasis and remodelling. Strong evidence has accumulated over the past two decades that bacterial components, including proteases and lipopolysaccharides, are key factors contributing to dysregulation of ECM homeostasis. It is of particular interest that activation of pro-MMP-2, which is constitutively expressed in PDL cells, is required for the fragmentation of cellular FN that is typically seen in periodontal disease (Miao et al., 2011). Thus, identifying factors that control MMP-2 expression or activation can help us better understand and modulate periodontal disease pathogenesis.

T. denticola is a member of a very complex microbiota involved in the pathogenesis of periodontal diseases. MMP-2 production and activation is a major event in the pathogenesis of these diseases via its role in extracellular matrix destruction. The *T. denticola* dentilisin protease plays an important role in the upregulation and activation of MMP-2 in PDL cells (Miao et al., 2011; Miao et al., 2014), thereby promoting further ECM destruction and release of FN fragments, which have deleterious effects on the periodontal environment (Dai et al., 2005; Ghosh et al., 2008; Jee et al., 2004; Joo et al., 2008;

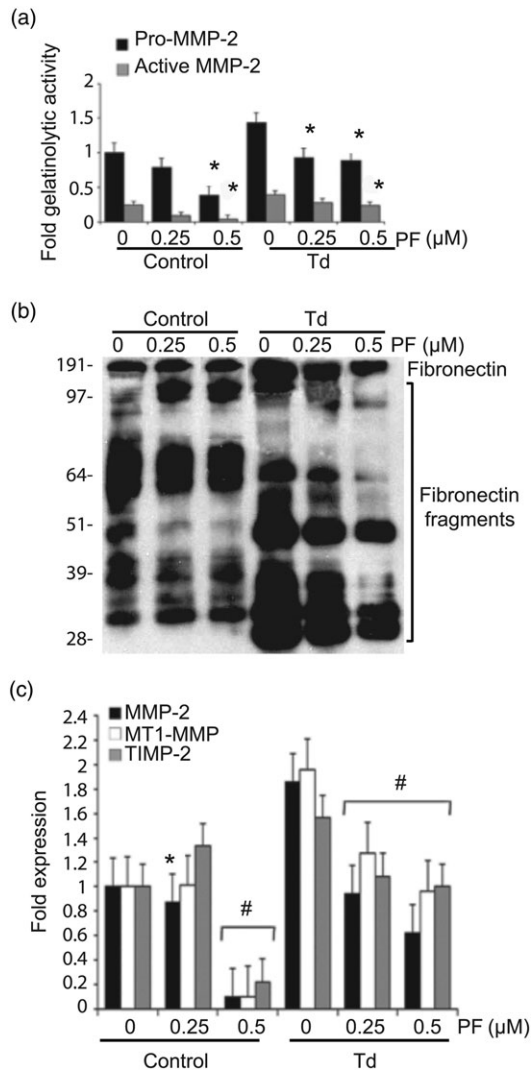


FIGURE 4 Inhibition of histone phosphorylation using PF-03814735 results in decreased expression and activation of MMP-2, MT1-MMP, and TIMP-2 and decreased fibronectin fragmentation in *Treponema denticola*-challenged and control periodontal ligament (PDL) cells. Cultured PDL cells were pretreated with 0.25 or 0.5 μM PF-03814735 (PF) for 2 days. The cells were then challenged with *T. denticola* (*Td*) at multiplicity of infection = 100 or media control for 2 hr and then incubated for 3 days. The conditioned medium and cell lysates were collected for zymography, Western blotting, and qRT-PCR. The experiments were repeated three times in triplicate. Data were analysed using one-way analysis of variance. (*) represents $p \leq .05$ compared to the "0" concentration in the same group. (#) represents $p \leq .001$ compared to the "0" concentration in the same group. (a) Densitometric analysis of pro-MMP-2 (72 kDa) and active MMP-2 (64 kDa) detected by gelatin zymography. The x axis represents different concentrations of histone kinase/phosphorylation inhibitor, PF-03814735 (PF) in the control and *Td* groups. The y axis represents fold-gelatinolytic activity of the pro-MMP-2 and active MMP-2 relative to unchallenged and untreated controls. (b) A representative immunoblot of PDL cell culture supernatants probed with a polyclonal anti-fibronectin antibody showing fibronectin fragmentation in conditioned medium from *Td*-challenged PDL cells, control and PF-treated. (c) Bar chart showing transcript levels of MMP-2, MT1-MMP, and TIMP-2 in the control and PF-treated PDL cells as determined by qRT-PCR. The y axis represents fold-expression level of each gene relative to unchallenged and untreated controls. The x axis represents different concentrations of PF in the control and *Td* groups

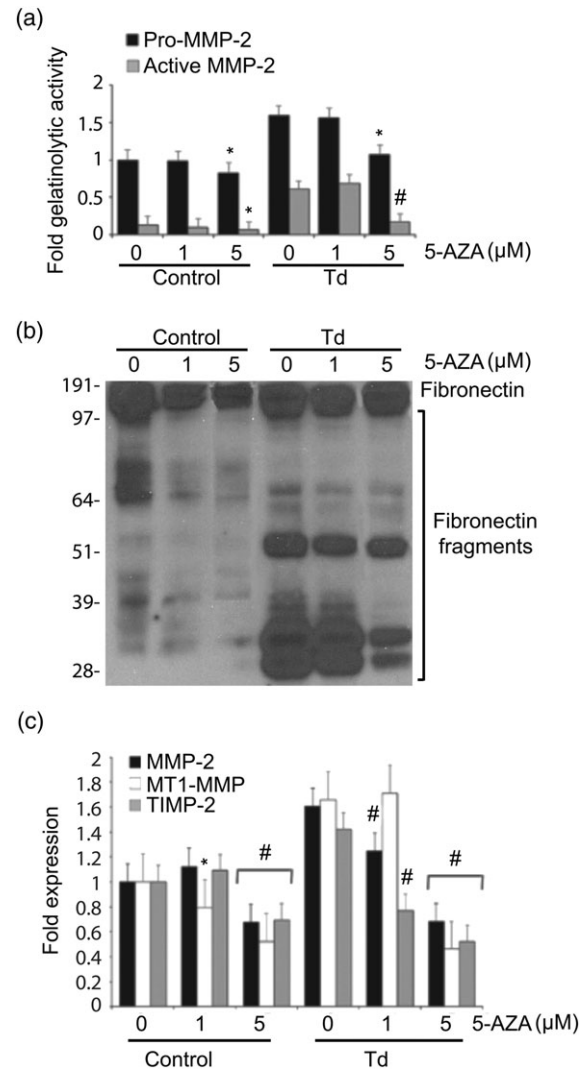


FIGURE 5 Inhibition of DNA methyltransferases using azacytidine (AZA) results in decreased expression and activation of MMP-2, MT1-MMP, and TIMP-2 and decreased fibronectin fragmentation in *Treponema denticola*-challenged and control periodontal ligament (PDL) cells. Cultured PDL cells were pretreated with 1 or 5 μM AZA for 4 days. The cells were then challenged with *T. denticola* (*Td*) at multiplicity of infection = 100 or media control for 2 hr and then incubated for 3 days. The conditioned medium and cell lysates were collected for zymography, Western blotting, and qRT-PCR. The experiments were repeated three times in triplicate. (a) Densitometric analysis of pro-MMP-2 (72 kDa) and active MMP-2 (64 kDa) detected by gelatin zymography. The x axis represents different concentrations of DNA methyltransferases inhibitor AZA in the control and *Td* groups. The y axis represents fold-gelatinolytic activity of the pro-MMP-2 and active MMP-2 relative to unchallenged and untreated controls. Data were analysed using one-way analysis of variance. (*) represents $p \leq .05$ compared to the "0" concentration in the same group. (#) represents $p \leq .001$ compared to the "0" concentration in the same group. (b) A representative immunoblot of PDL cell culture supernatants probed with a polyclonal anti-fibronectin antibody showing fibronectin fragmentation in conditioned medium from *Td*-challenged PDL cells, control and AZA-treated. (c) Bar chart showing transcript levels of MMP-2, MT1-MMP, and TIMP-2 in the control and AZA-treated PDL cells as determined by qRT-PCR. The y axis represents fold-expression level of each gene relative to unchallenged and untreated controls. The x axis represents different concentrations of AZA in the control and *Td* groups

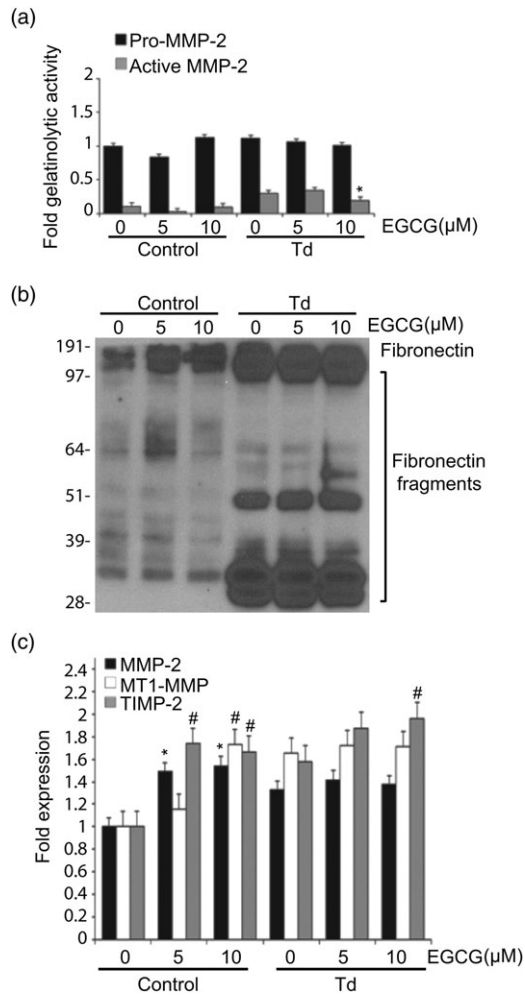


FIGURE 6 Inhibition of DNA methyltransferases using epigallocatechin gallate (EGCG) mediates a decrease in MMP-2 activation, an increase in TIMP-2 transcription, and minimal decrease in fibronectin fragmentation in *Td*-challenged periodontal ligament (PDL) cells. Cultured PDL cells were pretreated with 5 or 10 μM EGCG for 2 days before being challenged with *Treponema denticola* (*Td*) at multiplicity of infection = 100 or media control for 2 hr and then incubated for 3 days. The conditioned medium and cell lysates were collected for zymography, Western blotting, and qRT-PCR. The experiments were repeated three times in triplicate. Data were analysed using one-way analysis of variance. (*) represents $p \leq .05$ compared to the "0" concentration in the same group. (#) represents $p \leq .001$ compared to the "0" concentration in the same group. (a) Densitometric analysis of pro-MMP-2 (72 kDa) and active MMP-2 (64 kDa) detected by gelatin zymography. The left three lanes represent the control group (unchallenged PDL cells) and the right three lanes represent the *Td* group (*Td*-challenged PDL cells). The cells in both groups were treated with indicated concentrations of EGCG. The x axis represents different concentrations of EGCG in the control and *Td* groups. The y axis represents fold-gelatinolytic activity of pro-MMP-2 and active MMP-2 relative to unchallenged and untreated controls. (b) A representative immunoblot of PDL cell culture supernatants probed with a polyclonal anti-fibronectin antibody showing fibronectin fragmentation in conditioned medium from *Td*-challenged PDL cells, control and EGCG-treated. (c) Bar chart showing transcript levels of MMP-2, MT1-MMP, and TIMP-2 in the control and EGCG-treated PDL cells as determined by qRT-PCR. The y axis represents fold-expression level of each gene relative to unchallenged and untreated controls. The x axis represents different concentrations of EGCG in the control and *Td* groups

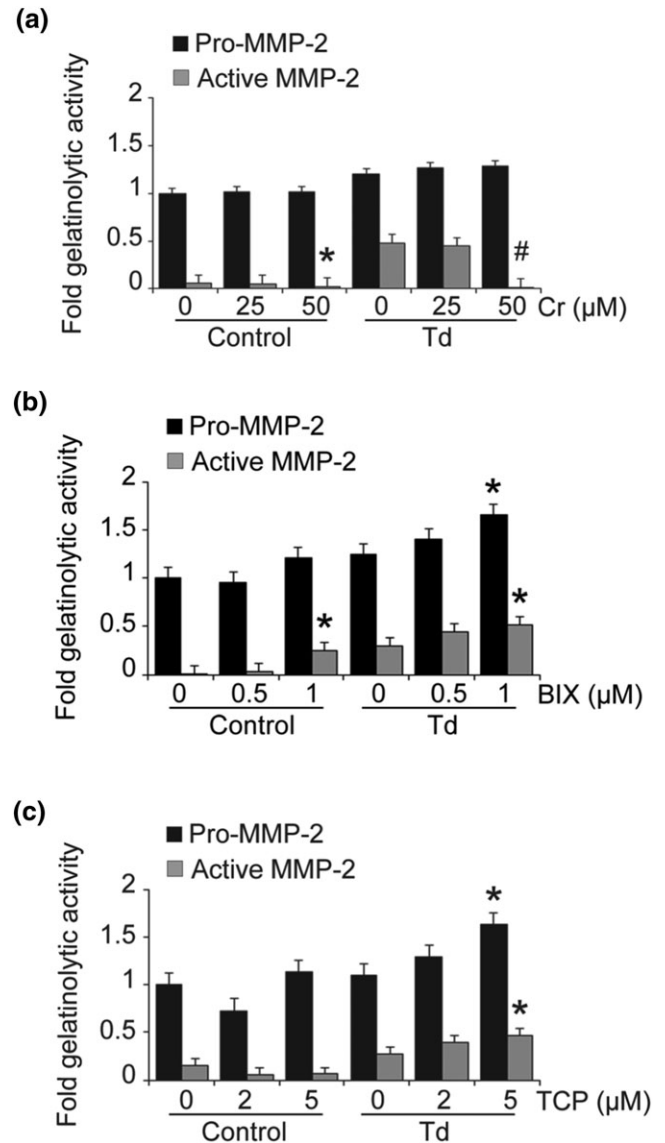


FIGURE 7 Inhibition of different chromatin modification enzymes mediate alterations in levels of expression and activation of MMP-2 in *Td*-challenged and control periodontal ligament (PDL) cell cultures. PDL cells were pretreated with enzyme inhibitors at the indicated concentrations as follows: (a) curcumin (histone acetyltransferase inhibitor) for 2 hr, (b) 0.5 μM and 1 μM BIX-01294 (histone methyltransferase inhibitor) for 2 days, and (c) 2 μM and 5 μM tranylcypromine/TCP (histone demethylase inhibitor) for 4 days. The cells were then challenged with *Treponema denticola* (*Td*) at multiplicity of infection = 100 or media control for 2 hr and then incubated for 3 days. The conditioned medium and cell lysates were collected for zymography. Shown are densitometric analyses of pro-MMP-2 (72 kDa) and active MMP-2 (64 kDa) detected by gelatin zymography. The x axis represents different concentrations of enzyme inhibitors in the control and *Td* groups. The y axis represents fold-gelatinolytic activity of the pro-MMP-2 and active MMP-2 relative to unchallenged and untreated controls. (a) Curcumin (Cr); (b) BIX-01294 (BIX); (c) tranylcypromine (TCP). The experiments were repeated three times in triplicate. Data were analysed using one-way analysis of variance. (*) represents $p \leq .05$ compared to the "0" concentration in the same group. (#) represents $p \leq .001$ compared to the "0" concentration in the same group

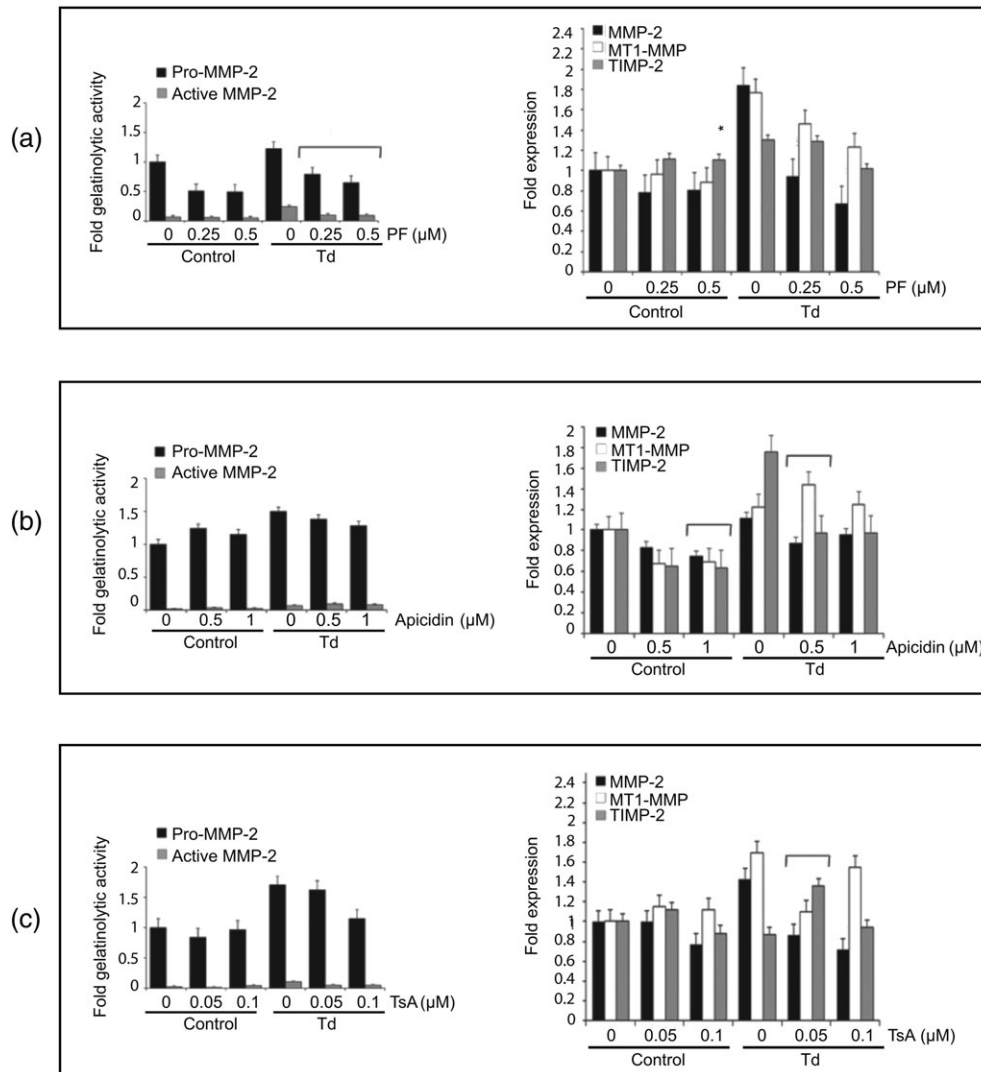


FIGURE 8 Inhibitors of histone phosphorylases and deacetylases reverse *Treponema denticola*-mediated increases in MMP-2 activity and expression in periodontal ligament (PDL) cells. Cultured PDL cells were challenged with *T. denticola* (*Td*) at multiplicity of infection = 100 or media control for 2 hr, incubated for 3 days in serum- and antibiotic-free MEM- α with daily culture medium refreshment, and then treated with the following enzyme inhibitors: (a) 0.25 or 0.5 μ M PF-03814735 (PF) for 2 days, (b) 0.5 or 1 μ M apicidin for 1 day, and (c) 0.05 or 0.1 μ M trichostatin A (TsA) for 4 days. The conditioned medium and cell lysates were collected for zymography, Western blotting, and qRT-PCR. The left portion of each panel shows densitometric analysis of pro-MMP-2 (72 kDa) and active MMP-2 (64 kDa) detected by gelatin zymography. The x axis represents different concentrations of the indicated inhibitor in the control and *Td*-challenged cultures. The y axis represents fold-gelatinolytic activity of the pro-MMP-2 and active MMP-2 relative to unchallenged and untreated controls. The right panel shows transcript levels of MMP-2, MT1-MMP, and TIMP-2 in the control and inhibitor-treated PDL cells as determined by qRT-PCR. The y axis represents fold-expression level of each gene relative to unchallenged and untreated controls. The x axis represents different concentrations of PF in the control and *Td* groups. The experiments were repeated three times in triplicate. Data were analysed using one-way analysis of variance. (*) represents $p \leq .05$ compared to the "0" concentration in the same group. (#) represents $p \leq .001$ compared to the "0" concentration in the same group

Joseph et al., 2010; Kapila et al., 1996; Kapila et al., 1999; Kapila, Wang, Dazin, Tafolla, & Mass, 2002; Miao et al., 2011; Miao et al., 2014; Pereira et al., 2014; Tafolla, Wang, Wong, Leong, & Kapila, 2005). The oral spirochete *Treponema lecithinolyticum* is also reported to activate MMP-2, as are *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* (Chang, Yang, Lai, Liu, & Hsieh, 2002; Choi et al., 2001; Song et al., 2003). The molecular mechanism(s) involved remain unstudied or unresolved for these species.

T. denticola dentilisin activity was present for several days in PDL cell conditioned medium, long after the brief (2 hr) challenge, washing and multiple changes of growth medium. This is consistent with our

prior reports (Miao et al., 2011; Miao et al., 2014) and is likely due to persistent adherence of *T. denticola* to PDL cells, though we cannot yet rule out potential downstream effects of previously documented uptake of *T. denticola* by PDL cells (Miao et al., 2014) and gingival epithelial cells (Inagaki, Kimizuka, Kokubu, Saito, & Ishihara, 2016; Jo, Baek, Shin, & Choi, 2014; Shin & Choi, 2012). Importantly, although dentilisin activity levels decreased steadily over time, MMP-2 and its activating complex, MT1-MMP and TIMP-2, were clearly expressed and activated through the 12th day of culture. These data confirm our previous results that dentilisin is an important factor in the activation of pro-MMP-2 (Miao et al., 2011). Additionally, in

agreement with our earlier findings (Miao et al., 2014), the current data show that *T. denticola* chronically activates MMP-2, in concert with MT1-MMP and TIMP-2 expression up to 12 days. Importantly, degradation of cellular FN in PDL cell cultures is dependent on MMP-2 activation, which is the result of *T. denticola* dentilisin activity (Miao et al., 2011). The persistent FN fragmentation in these samples may help explain the chronicity of tissue destruction mediated by bacterial proteases during periodontal disease pathogenesis.

Previous studies showed that *T. denticola* has the ability to adhere to and be internalised by several different host cells, including gingival epithelial cells, PDL cells, and polymorphonuclear leukocytes (Ding et al., 1997; Konermann et al., 2012; Miao et al., 2014; Shin & Choi, 2012). It should be noted that, unlike other oral pathogens, such as *P. gingivalis* that exhibits a cellular-invasive phenotype (Lamont et al., 1995), there is no reported evidence that *T. denticola* survives in the intracellular environment. Taken in aggregate, these results and the findings from this study support the concept that the alteration of the PDL cells toward a destructive phenotype is a consequence of exposure to *T. denticola*. These data are consistent with evidence from other inflammatory diseases pointing toward an epigenetic role in the pathogenic process. We hypothesized that epigenetic mechanisms may imprint the periodontium and set in motion the process of chronic periodontal tissue destruction.

Mechanisms of epigenetic modifications include histone acetylation, histone methylation, DNA methylation, positioning of histone variants, and gene regulation by noncoding microRNAs (miRNAs; Bayarsaihan, 2016; Herceg, 2016; Perkins, Patel, Blanco, & Vogel, 2016). Several enzymes are involved in these mechanisms including HATs, HDACs, HMTs, HDMs, histone phosphorylases, and DNMTs.

Perturbing the balance between acetylation/deacetylation and methylation/demethylation is profoundly associated with numerous diseases including developmental abnormalities and cancer and chronic inflammatory conditions (Barros & Offenbacher, 2009; Bird, 2002). Epigenetic modifications are potentially reversible; therefore, a thorough understanding of these changes may identify new therapeutic targets for disease management. Using gene arrays that target chromatin modification enzymes, our data provide the first evidence that *T. denticola* mediates downregulation of many of these enzymes in PDL cells. In similar studies, *P. gingivalis* lipopolysaccharides were shown to downregulate many chromatin modification enzymes in cultured oral keratinocytes (de Camargo Pereira et al., 2013) and change the expression of multiple miRNAs in PDL cells (Du et al., 2016). However, because *T. denticola*-dependent MMP-2 activation requires dentilisin proteolytic activity (Miao et al., 2011), it is likely that the molecular mechanisms responsible for this downregulation differ from those driven by *P. gingivalis* lipopolysaccharides. Specific mechanisms by which *T. denticola* or its dentilisin protease may regulate epigenetic enzyme expression are under study in our laboratories.

The association between *T. denticola* and periodontitis has been reported in numerous studies (Asai, Jinno, Igarashi, Ohyama, & Ogawa, 2002; Sakamoto, Takeuchi, Umeda, Ishikawa, & Benno, 2001; Simonson et al., 1988; Yoshida et al., 2004). Consistent with the existing literature, we found that *T. denticola* levels were significantly elevated in human tissue specimens from periodontally diseased sites compared with healthy sites, as measured by qPCR normalised to

human glyceraldehyde-3-phosphate dehydrogenase (GAPDH; data not shown). To date, studies of the role of epigenetic modifications in periodontal disease have focused on DNA methylation patterns in genes involved in inflammatory responses (Barros & Offenbacher, 2014). The present study used gene array approaches to examine healthy and diseased periodontal tissues, revealing for the first time significant alterations in the expression of several chromatin modification enzymes in diseased tissues. Although expression levels of some enzymes (such as Aurora Kinase B) differed in the gene array of the PDL cells compared to the tissues, this likely reflects the fact that tissues exhibit the net expression of diverse cell types that comprise the periodontium, as well as the net effects of the diverse oral microbiome on the periodontium, and net effects of other epigenetic effectors, including smoking and medications.

Different inhibitors show promise in reversing epigenetic changes in the context of our study and in other reports. Chronic *T. denticola*-mediated MMP-2 expression and activation were decreased in *T. denticola*-challenged PDL cells either pretreated or post-treated with inhibitors of histone phosphorylases, HDACs, and DNMTs. This indicates that *T. denticola* induces epigenetic changes mediated by histone phosphorylation, histone deacetylation, or DNA methylation pathways. Specifically, pretreatment or post-treatment inhibition of histone phosphorylation in PDL cells using PF-03814735 significantly prevented or reversed the *T. denticola*-mediated increase in MMP-2 and the MT1-MMP/TIMP-2 complex. This study is the first to show the effect of inhibition of histone phosphorylation on MMP-2 expression in PDL cells. In a broader context, PF has been used in Phase I clinical trials for the treatment of advanced solid tumours (Jani et al., 2010; Schoffski et al., 2011).

Inhibition of HDACs using apicidin and/or trichostatin was also effective in preventing or reversing the *T. denticola*-mediated effects on the expression of MMP-2 in PDL cells. Similar data were obtained by other studies investigating the effect of trichostatin on MMP-2 expression in murine fibroblasts (Ailenberg & Silverman, 2002). Trichostatin was further shown to decrease MMP-2 and MMP-9 expression in murine heart cells (Mani et al., 2015) and in human oesophageal squamous cell carcinoma cells (Wang et al., 2013). Trichostatin, in combination with SAHA, was reported to inhibit the respiratory syncytial virus-mediated increase in HDAC2 expression with resultant decrease in airway inflammation and oxidative stress in vivo (Feng et al., 2016). Apicidin was also reported to inhibit the expression of MMP-2 in different cancer cells (Ahn et al., 2012; Kim, Son, Kim, In Park, & Moon, 2000; Park et al., 2011). On the other hand, inhibition of DNMTs using AZA showed a reduction in the expression of MMP-2 and its activating complex, whereas the use EGCG was not effective in this process.

Our results are consistent with those in other studies reporting that AZA causes a downregulation of MMP-2 and MMP-9 expression in cultured oesophageal squamous cell carcinoma cells (Liu, Sang, Hou, Zhang, & Shan, 2014) and breast cancer cells (Chang et al., 2014), respectively. AZA was approved by the U.S. Food and Drug Administration for treatment of myelodysplastic syndromes (Nebbio, Carafa, Benedetti, & Altucci, 2012). It was also investigated in many clinical trials for treatment of several disorders, including

haematological and neoplastic disorders (Chen, Huang, & Zha, 2012; Liu et al., 2005; Mirza, Sharma, Pandya, & Ralhan, 2010). Regarding the effect of EGCG on MMP-2 expression in our study, the data are inconsistent with other studies, which reported the ability of EGCG to inhibit MMP-2 in different cancer cells (Chang et al., 2014, Nowakowska & Tarasiuk, 2016). This inconsistency may be due to the use of different cell types, different concentrations of EGCG, or both. Concentrations higher than 10 μ M were toxic to PDL cells as examined by cell proliferation and cytotoxicity assays (data not shown). Due to the weak effect of EGCG on the expression of MMP-2, we did not test its effects in a postchallenge scenario.

Negative consequences of using epigenetic enzyme inhibitors are possible, as seen in the current study. Specifically, some inhibitors, namely, those inhibiting HDM with TCP and those inhibiting HMT with BIX 01295, further increased MMP-2 expression and activation. Although TCP and BIX are thought to mediate opposite actions, namely, BIX inhibits histone methylation and TCP promotes histone methylation, both increased MMP-2 expression and activation. Thus, due to these undesirable effects, BIX and TCP were not evaluated further in postchallenge scenarios. There are no previous studies investigating their effect on expression of MMPs. However, Pereira et al (2014) showed that increases in MMP-2 expression were stimulated by decreases in histone methylation in the context of the MMP-2 promoter. Additionally, in another study, the MMP-1, 3, 9, and 13 genes were shown to be actively transcribed in rheumatoid arthritis-derived synovial fibroblasts, and this transcription correlated with an elevation in H3K4me3 and suppression of H3K27me3 in the MMP promoter genes (Araki et al., 2016). These studies, which both showed increases in MMP-2 expression despite different degrees of histone methylation, can be explained by the fact that activation or repression of gene expression by histone modifications/methylations depends on the type of lysine being modified and the degree of its methylation. For example, H3K4me, H3K36me, or H3K79me are associated with transcriptional activation (Barski et al., 2007; Guenther, Levine, Boyer, Jaenisch, & Young, 2007; Jenuwein & Allis, 2001; Koch et al., 2007; Kouzarides, 2007; Zhang & Reinberg, 2001), whereas H3K9me, H3K27me, or H4K20me are implicated in gene repression (Barski et al., 2007; Jenuwein & Allis, 2001; Kouzarides, 2007; Nakayama, Rice, Strahl, Allis, & Grewal, 2001; Talbert & Henikoff, 2006).

The HAT inhibitor curcumin also increased the expression of MMP-2 but significantly inhibited its activation. The effect of curcumin in decreasing MMP-2 and MMP-9 expression levels was reported in cancer cells, such as squamous cell carcinoma and osteoclastoma (Cao, Liu, Xu, Xu, & Feng, 2015; Lee et al., 2015). Additionally, in vivo studies in humans and animals demonstrated curcumin's effectiveness in decreasing the severity of periodontal diseases (Bakir et al., 2016; Elburki et al., 2014; Elburki et al., 2016; Nagasri et al., 2015). Curcumin has been investigated in many clinical trials for treatment of several disorders, such as ulcerative colitis (Baliga et al., 2012), breast cancer (Nagaraju et al., 2012), pancreatic cancer (Veeraraghavan et al., 2011), and diabetes (Abdel Aziz et al., 2012). Additional investigation into the role of curcumin in MMP activation and regulation in periodontal diseases is further warranted, given these reports and the current study findings.

In summary, *T. denticola* plays a key role in the transcriptional regulation of MMP-2 and its activating complex MT1-MMP/TIMP-2 in PDL cells. *T. denticola* also mediates alterations of chromatin modification enzyme expression in PDL cells, and an array of epigenetic modifications are associated with periodontally diseased tissues. Furthermore, inhibition of enzymes that mediate epigenetic modifications can prevent *T. denticola*-mediated increases in MMP-2, MT1-MMP, and TIMP-2 in PDL cells. These inhibitors can also reverse the *T. denticola*-mediated effects on MMP-2 and its activating complex in PDL cells. These data indicate that *T. denticola* mediates its effects on MMP-2 activation through epigenetic modifications in these cells. This knowledge can be useful as a first step toward the development of novel targeted therapeutics for the treatment of periodontal diseases.

4 | EXPERIMENTAL PROCEDURES

4.1 | PDL cell culture

As described previously, the primary culture of PDL cells was obtained via the direct cell outgrowth method by isolating cells from the PDL tissue around the middle third of extracted healthy human teeth (Scanlon, Marchesan, Soehren, Matsuo, & Kapila, 2011; Tanaka et al., 2011). Cells were maintained in minimal essential medium- α (MEM- α) augmented with 10% fetal bovine serum, 1% penicillin/streptomycin (P/S), and 1% amphotericin B (Gibco, Grand Island, NY, USA) in a humid atmosphere with 95% air and 5% CO₂ at 37 °C. Cell outgrowths were passaged when they reached confluency. Cells passaged three to six times were used for experimentation. The cell counting kit-8 (Dojindo, Rockville, MD, USA) was used for assaying PDL cell proliferation and cytotoxicity at different time intervals in response to different concentrations of the inhibitors used in the study (data not shown). Protocols involving the collection and use of human teeth and PDL cells/tissue were approved by the Health Sciences Institutional Review Board of the University of Michigan.

4.2 | Human periodontal tissues

Periodontal tissues were obtained by collecting the tissues around the coronal third of the roots of extracted periodontally involved teeth and healthy teeth, both from the posterior mandibular region. Periodontal status was diagnosed clinically by testing for bleeding on probing of the gingival sulcus and periodontal pocket depth measurements. The samples were collected from six different periodontitis patients and six healthy subjects. The collection and use of human teeth and PDL tissues was approved by the Health Sciences Institutional Review Board of the University of Michigan.

4.3 | Culture of *T. denticola*

T. denticola ATCC 35405 was grown as described previously under anaerobic conditions at 37 °C in new oral spirochete broth medium (Fenno, 2005; Haapasalo, Singh, McBride, & Uitto, 1991). Purity of spirochete cultures was confirmed by darkfield microscopy prior to use in experiments.

4.4 | Challenge of PDL cells with *T. denticola*

PDL cells were prepared in MEM- α free of serum and antibiotics. The bacteria in broth culture were collected by centrifugation, and then resuspended in serum- and antibiotic-free MEM- α to an optical density of 0.1 at 600 nm, such that the cellular density was approximately 2.4×10^8 cells/ml.

T. denticola in serum- and antibiotic-free MEM- α was added to the test group of PDL cells (*T. denticola* group) at a multiplicity of infection (MOI) = 100, whereas only MEM- α was added to the control group. Both groups were then incubated for 2 hr at 37 °C in 5% CO₂-containing air. After the 2-hr challenge, PDL cells were washed three times with PBS and incubated for the planned time periods in serum- and antibiotic-free MEM- α with daily medium changes as described previously (Miao et al., 2011). Subsequently, the culture conditioned medium and cell lysates were collected, RNA was extracted from the cell lysates, and all samples were stored at -80 °C for further investigations.

4.5 | Prechallenge inhibition of epigenetic chromatin modification enzymes in PDL cells

Different inhibitors of epigenetic chromatin modification enzymes (listed in Table 1) were obtained and prepared according to the manufacturer's instructions and then brought to the desired concentrations via dilution in serum- and antibiotic-free MEM- α . With the exception of tranilcypromine (EMD Millipore, Temecula, CA, USA), all enzyme inhibitors were purchased from Sigma Aldrich (St. Louis, MO, USA).

PDL cells were treated with the indicated concentrations of the enzyme inhibitors either as single agents or in combinations and incubated for the indicated times with daily culture medium replacement. The cells were then challenged with *T. denticola* at an MOI of 1:100 for 2 hr, washed, and incubated for 3 days in serum- and antibiotic-free MEM- α with daily culture medium refreshment. At the end of this incubation period, conditioned culture medium and cell lysates were harvested and stored at -80 °C for further investigations.

4.6 | Postchallenge inhibition of epigenetic chromatin modification enzymes in PDL cells

For the postchallenge experiments, the PDL cells were first challenged with *T. denticola* as described before and then treated with the indicated concentrations of the inhibitors either as single agents or in combinations for certain periods. At the end of the treatment period, the conditioned culture medium and cell lysates were harvested and stored at -80 °C for further investigations. All experiments were repeated at least three times, and each experiment was performed in triplicate.

4.7 | Gelatin zymography

Culture supernatants were concentrated approximately tenfold in Amicon centrifugal concentrators (10,000-molecular-weight cut-off; Millipore), and total protein concentration was measured using the

BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) normalised to an albumin standard. Equivalent protein concentrations from each sample were mixed with nonreducing sample buffer (0.25 M tris-base, 40% glycerol, 0.8% sodium dodecyl sulfate (SDS), and 0.05% bromophenol blue stain in distilled deionised water/ddH₂O at pH 6.8) and loaded into 8% polyacrylamide gels copolymerised with 0.4% SDS and 0.2% gelatin. Samples were electrophoretically resolved on gelatin-containing gels at 125 V for 110 min at 4 °C. Gels were then washed twice for 15 min under continuous agitation using renaturation/washing buffer (2.5% v/v Triton-X100 and 0.05 M Tris-base in ddH₂O at pH 7.5) to eliminate SDS and promote the renaturation of MMP enzymes. Subsequently, gels were incubated in developing/incubation buffer (0.05 M Tris-base, 0.15 M sodium chloride, 0.01 M calcium chloride, and 0.02% sodium azide in ddH₂O at pH 7.5) for 30 min under agitation, and then the buffer was replaced and gels incubated for 16–20 hr at 37 °C. After that, gels were stained using filtered Coomassie Brilliant blue stain for 1 or 2 hr under agitation. Destaining of the gels was performed using a methanol/acetic acid destaining buffer (40% methanol and 10% acetic acid in ddH₂O) until the bands on the gel appeared clear. Zymograms were scanned, and the densitometry of the gelatinolytic activity represented by the clear bands was analysed using ImageJ software (NIH, USA). Brightness and contrast levels of zymogram images were slightly adjusted for publication only.

4.8 | Immunoblotting

Equivalent protein samples consisting of tenfold concentrates of PDL cell culture conditioned media were standardised as described above, subjected to standard SDS-PAGE (4–12% polyacrylamide gels; Invitrogen, Carlsbad, CA, USA) and transferred to polyvinylidene difluoride (PVDF) membranes using standard techniques. The membranes were exposed to a rabbit polyclonal anti-FN IgG (Santa Cruz, Dallas, TX, USA) primary antibody diluted 1:2000 in Tris-buffered saline, 0.1% Tween 20 (TBST) solution for 2 hr at room temperature followed by a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Santa Cruz, Dallas, TX, USA) diluted 1:10,000 in TBST for 1 hr at room temperature with agitation. Western blots using anti-GAPDH antibodies were used to further confirm equal protein loading (data not shown). Blots were developed using the SuperSignal® West Pico kit (ThermoFischer Scientific, Pittsburgh, PA, USA) and scanned for digitization.

4.9 | Quantitative reverse transcriptase polymerase chain reaction

qRT-PCR was performed to assess the transcriptional levels of MMP-2, MT1-MMP, and TIMP-2 in PDL cells. Cell lysates were collected, and the RNA was extracted and purified using the RNeasy® mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Reverse transcription of the RNA into cDNA was then performed using the SuperScript™ II RT kit (Invitrogen, Carlsbad, CA, USA). The cDNA was then amplified by qPCR using the TaqMan® Universal PCR Master Mix (Invitrogen, Carlsbad, CA, USA) on a ViiA™ 7 Applied Biosystems® PCR system. The following TaqMan® human probes were used: MMP-2 (Hs01548727_m1), MT1-MMP

(Hs01037003_g1), TIMP-2 (Hs00234278_m1), and GAPDH (Hs03929097_g1; Invitrogen, Carlsbad, CA, USA). The cycle threshold (C_t) values were obtained and analysed, and the quantitative expression of target genes in challenged PDL cells was normalised to GAPDH and compared to the control cells using the $2^{-\Delta\Delta C_T}$ method (Livak & Schmittgen, 2001), applying a minimum twofold change in expression as the cut-off.

Similar methodology was utilised to assess the levels of MMP-2 transcription and the levels of *T. denticola* in human periodontal tissue samples. Periodontal tissue specimens were collected by scraping the most coronal portion of the tissue around the roots of extracted healthy and periodontally diseased teeth. Total RNA was extracted from healthy and diseased periodontal tissue specimens, and cDNA was generated. Target genes were amplified using TaqMan® Universal PCR Master Mix (Invitrogen, Carlsbad, CA, USA). Tissue sample human genome content was normalised using a custom TaqMan® primer/probe set for GAPDH (Hs03929097_g1; Invitrogen, Carlsbad, CA, USA). *T. denticola* 16S rRNA was amplified in parallel using the SYBR® Green PCR Master Mix (Invitrogen, Carlsbad, CA, USA) with the following primer set: 16SrRNA-987F AGGGATATGGCAGCGTAGCA and 16SrRNA-1077R TTGCGGGACTTAACCAACA.

4.10 | Quantitative reverse transcription (qRT-PCR) gene micro-array

qRT-PCR arrays were used to assess the transcriptional levels of the main epigenetic chromatin modification enzymes in cultured PDL cells and in periodontal tissues using the RT² Profiler PCR Array kit (Qiagen, Valencia, CA, USA) to assay transcription of the genes listed in Table S1. Periodontal tissue specimens were collected by scraping the most coronal portion of the tissue around the roots of extracted healthy and periodontally diseased teeth. RNA was extracted and purified from PDL cell cultures using the RNeasy® Mini kit, whereas extraction and purification of RNA from periodontal tissues was achieved using the RNeasy® Protect Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Reverse transcription of RNA into cDNA was performed using the RT² First Strand Kit (Qiagen, Valencia, CA, USA). The target genes were then amplified using the RT² Profiler PCR Array kit and RT² SYBR® Green Master mix (Qiagen, Valencia, CA, USA) on a ViiA™ 7 Applied Biosystems® PCR system. Applying a minimum twofold change in expression as the cut-off, C_t values were analysed, and the quantitative expression of the genes of interest in *T. denticola*-challenged PDL cells was normalised to supplied house-keeping genes, β -actin, GAPDH, β -2-microglobulin, hypoxanthine phosphoribosyltransferase-1, and large ribosomal protein-P0, and then compared to expression levels in unchallenged PDL cells and the healthy tissues (Livak & Schmittgen, 2001).

4.11 | Statistical analysis

The data were analysed using the statistical software SPSS® v.22 (IBM, Armonk, NY, USA). Results were evaluated by a one-way analysis of variance when comparing more than two groups, whereas the Student's *t* test was used when comparing two groups. $p \leq .05$ was

considered significant, whereas $p \leq .001$ was considered highly significant.

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REFERENCES

- Abdel Aziz, M. T., El-Asmar, M. F., El-Ibrashy, I. N., Rezaq, A. M., Al-Malki, A. L., Wassef, M. A., ... Morsi, H. M. (2012). Effect of novel water soluble curcumin derivative on experimental type- 1 diabetes mellitus (short term study). *Diabetology and Metabolic Syndrome*, 4, 30.
- Achour, M., Mousli, M., Alhosin, M., Ibrahim, A., Peluso, J., Muller, C. D., ... Bronner, C. (2013). Epigallocatechin-3-gallate up-regulates tumor suppressor gene expression via a reactive oxygen species-dependent down-regulation of UHRF1. *Biochemical and Biophysical Research Communications*, 430, 208–212.
- Ahn, M. Y., Kang, D. O., Na, Y. J., Yoon, S., Choi, W. S., Kang, K. W., ... Kim, H. S. (2012). Histone deacetylase inhibitor, apicidin, inhibits human ovarian cancer cell migration via class II histone deacetylase 4 silencing. *Cancer Letters*, 325, 189–199.
- Ailenberg, M., & Silverman, M. (2002). Trichostatin A—histone deacetylase inhibitor with clinical therapeutic potential—is also a selective and potent inhibitor of gelatinase A expression. *Biochemical and Biophysical Research Communications*, 298, 110–115.
- Andia, D. C., de Oliveira, N. F., Casarin, R. C., Casati, M. Z., Line, S. R., & de Souza, A. P. (2010). DNA methylation status of the IL8 gene promoter in aggressive periodontitis. *Journal of Periodontology*, 81, 1336–1341.
- Araki, Y., Tsuzuki Wada, T., Aizaki, Y., Sato, K., Yokota, K., Fujimoto, K., ... Mimura, T. (2016). Histone methylation and STAT-3 differentially regulate interleukin-6-induced matrix metalloproteinase gene activation in rheumatoid arthritis synovial fibroblasts. *Arthritis & Rheumatology*, 68, 1111–1123.
- Aresu, L., Benali, S., Garbisa, S., Gallo, E., & Castagnaro, M. (2011). Matrix metalloproteinases and their role in the renal epithelial mesenchymal transition. *Histology and Histopathology*, 26, 307–313.
- Armitage, G. C. (2004). Periodontal diagnoses and classification of periodontal diseases. *Periodontology* 2000, 34, 9–21.
- Armitage, G. C. (2008). Challenges in diagnosis and classification of periodontal diseases and conditions. *Zhonghua Kou Qiang Yi Xue Za Zhi*, 43, 260–263.
- Asai, Y., Jinno, T., Igarashi, H., Ohyama, Y., & Ogawa, T. (2002). Detection and quantification of oral treponemes in subgingival plaque by real-time PCR. *Journal of Clinical Microbiology*, 40, 3334–3340.
- Azevedo, A., Prado, A. F., Antonio, R. C., Issa, J. P., & Gerlach, R. F. (2014). Matrix metalloproteinases are involved in cardiovascular diseases. *Basic & Clinical Pharmacology & Toxicology*, 115, 301–314.
- Bakir, B., Yetkin Ay, Z., Buyukbayram, H. I., Kumbul Doguc, D., Bayram, D., Candan, I. A., & Uskun, E. (2016). The effect of curcumin treatment on systemic Th17 response; gingival expression of IL-17 and retinoic acid receptor-related orphan receptor γ ; and alveolar bone loss in experimental periodontitis. *Journal of Periodontology*, 1–17.
- Balasubramanyam, K., Varier, R. A., Altaf, M., Swaminathan, V., Siddappa, N. B., Ranga, U., & Kundu, T. K. (2004). Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone

- acetyltransferase-dependent chromatin transcription. *The Journal of Biological Chemistry*, 279, 51163–51171.
- Baliga, M. S., Joseph, N., Venkataranganna, M. V., Saxena, A., Ponemone, V., & Fayad, R. (2012). Curcumin, an active component of turmeric in the prevention and treatment of ulcerative colitis: Preclinical and clinical observations. *Food & Function*, 3, 1109–1117.
- Barros, S. P., & Offenbacher, S. (2009). Epigenetics: Connecting environment and genotype to phenotype and disease. *Journal of Dental Research*, 88, 400–408.
- Barros, S. P., & Offenbacher, S. (2014). Modifiable risk factors in periodontal disease: Epigenetic regulation of gene expression in the inflammatory response. *Periodontology 2000*, 64, 95–110.
- Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., ... Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. *Cell*, 129, 823–837.
- Bauden, M., Tassidis, H., & Ansari, D. (2015). In vitro cytotoxicity evaluation of HDAC inhibitor apicidin in pancreatic carcinoma cells subsequent time and dose dependent treatment. *Toxicology Letters*, 236, 8–15.
- Bayarsaihan, D. (2011). Epigenetic mechanisms in inflammation. *Journal of Dental Research*, 90, 9–17.
- Bayarsaihan, D. (2016). Epigenetic mechanisms involved in modulation of inflammatory diseases. *Current Opinion in Clinical Nutrition and Metabolic Care*, 19, 263–269.
- Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes & Development*, 16, 6–21.
- Borkham-Kamphorst, E., Alexi, P., Tihaa, L., Haas, U., & Weiskirchen, R. (2015). Platelet-derived growth factor-D modulates extracellular matrix homeostasis and remodeling through TIMP-1 induction and attenuation of MMP-2 and MMP-9 gelatinase activities. *Biochemical and Biophysical Research Communications*, 457, 307–313.
- Brew, K., & Nagase, H. (2010). The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity. *Biochimica et Biophysica Acta*, 1803, 55–71.
- Butler, G. S., Butler, M. J., Atkinson, S. J., Will, H., Tamura, T., Schade van Westrum, S., ... Murphy, G. (1998). The TIMP2 membrane type 1 metalloproteinase “receptor” regulates the concentration and efficient activation of progelatinase A. A kinetic study. *The Journal of Biological Chemistry*, 273, 871–880.
- Cao, F., Liu, T., Xu, Y., Xu, D., & Feng, S. (2015). Curcumin inhibits cell proliferation and promotes apoptosis in human osteoclastoma cell through MMP-9, NF-kappaB and JNK signaling pathways. *International Journal of Clinical and Experimental Pathology*, 8, 6037–6045.
- Chang, C. W., Hsieh, Y. H., Yang, W. E., Yang, S. F., Chen, Y., & Hu, D. N. (2014). Epigallocatechingallate inhibits migration of human uveal melanoma cells via downregulation of matrix metalloproteinase-2 activity and ERK1/2 pathway. *BioMed Research International*, 2014, 141582.
- Chang, H. W., Wang, H. C., Chen, C. Y., Hung, T. W., Hou, M. F., Yuan, S. S., ... Tseng, C. N. (2014). 5-azacytidine induces anoikis, inhibits mammosphere formation and reduces metalloproteinase 9 activity in MCF-7 human breast cancer cells. *Molecules*, 19, 3149–3159.
- Chang, J., Varghese, D. S., Gillam, M. C., Peyton, M., Modi, B., Schiltz, R. L., ... Martinez, E. D. (2012). Differential response of cancer cells to HDAC inhibitors trichostatin A and depsipeptide. *British Journal of Cancer*, 106, 116–125.
- Chang, Y. C., Yang, S. F., Lai, C. C., Liu, J. Y., & Hsieh, Y. S. (2002). Regulation of matrix metalloproteinase production by cytokines, pharmacological agents and periodontal pathogens in human periodontal ligament fibroblast cultures. *Journal of Periodontal Research*, 37, 196–203.
- Charitaki, E., Kastiris, E., Petraki, C., Liapis, K., Adamidis, K., Apostolou, T., ... Dimopoulos, M. A. (2016). Glomerular expression of matrix metalloproteinases in AL-amyloidosis and association with renal function at the time of kidney biopsy. *Clinical Nephrology*, 85, 44–54.
- Chen, W. F., Huang, F., & Zha, J. (2012). The comparison of short-term efficacies between decitabine and HA regimen for MDS/AML patients. *Zhonghua Xue Ye Xue Za Zhi*, 33, 143–144.
- Chernov, A. V., Sounni, N. E., Remacle, A. G., & Strongin, A. Y. (2009). Epigenetic control of the invasion-promoting MT1-MMP/MMP-2/TIMP-2 axis in cancer cells. *The Journal of Biological Chemistry*, 284, 12727–12734.
- Chi, B., Qi, M., & Kuramitsu, H. K. (2003). Role of dentilis in *Treponema denticola* epithelial cell layer penetration. *Research in Microbiology*, 154, 637–643.
- Choi, B. K., Jung, J. H., Suh, H. Y., Yoo, Y. J., Cho, K. S., Chai, J. K., & Kim, C. K. (2001). Activation of matrix metalloproteinase-2 by a novel oral spirochetal species *Treponema lecithinolyticum*. *Journal of Periodontology*, 72, 1594–1600.
- Choi, B. K., Paster, B. J., Dewhirst, F. E., & Gobel, U. B. (1994). Diversity of cultivable and uncultivable oral spirochetes from a patient with severe destructive periodontitis. *Infection and Immunity*, 62, 1889–1895.
- Dai, R., Iwama, A., Wang, S., & Kapila, Y. L. (2005). Disease-associated fibronectin matrix fragments trigger anoikis of human primary ligament cells: p53 and c-myc are suppressed. *Apoptosis*, 10, 503–512.
- de Camargo Pereira, G., Guimaraes, G. N., Planello, A. C., Santamaria, M. P., de Souza, A. P., Line, S. R., & Marques, M. R. (2013). *Porphyromonas gingivalis* LPS stimulation downregulates DNMT1, DNMT3a, and JMJD3 gene expression levels in human HaCaT keratinocytes. *Clinical Oral Investigations*, 17, 1279–1285.
- Ding, Y., Haapasalo, M., Kerosuo, E., Lounatmaa, K., Kotiranta, A., & Sorsa, T. (1997). Release and activation of human neutrophil matrix metallo- and serine proteinases during phagocytosis of *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Treponema denticola*. *Journal of Clinical Periodontology*, 24, 237–248.
- Du, A., Zhao, S., Wan, L., Liu, T., Peng, Z., Zhou, Z., ... Fang, H. (2016). MicroRNA expression profile of human periodontal ligament cells under the influence of *Porphyromonas gingivalis* LPS. *Journal of Cellular and Molecular Medicine*, 20, 1329–1338.
- Ejeil, A. L., Igondjo-Tchen, S., Ghomrasseni, S., Pellat, B., Godeau, G., & Gogly, B. (2003). Expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in healthy and diseased human gingiva. *Journal of Periodontology*, 74, 188–195.
- Elburki, M. S., Moore, D. D., Terezakis, N. G., Zhang, Y., Lee, H. M., Johnson, F., & Golub, L. M. (2016). A novel chemically modified curcumin reduces inflammation-mediated connective tissue breakdown in a rat model of diabetes: Periodontal and systemic effects. *Journal of Periodontal Research*.
- Elburki, M. S., Rossa, C., Guimaraes, M. R., Goodenough, M., Lee, H. M., Curylofo, F. A., ... Golub, L. M. (2014). A novel chemically modified curcumin reduces severity of experimental periodontal disease in rats: Initial observations. *Mediators of Inflammation*, 2014, 959471.
- Ellen, R. P., Dawson, J. R., & Yang, P. F. (1994). *Treponema denticola* as a model for polar adhesion and cytopathogenicity of spirochetes. *Trends in Microbiology*, 2, 114–119.
- Ellen, R. P., & Galimanas, V. B. (2005). Spirochetes at the forefront of periodontal infections. *Periodontology 2000*, 38, 13–32.
- Endres, M., Kneitz, S., Orth, M. F., Perera, R. K., Zerneck, A., & Butt, E. (2016). Regulation of matrix metalloproteinases (MMPs) expression and secretion in MDA-MB-231 breast cancer cells by LIM and SH3 protein 1 (LASP1). *Oncotarget*.
- Feng, Q., Su, Z., Song, S., Chiu, H., Zhang, B., Yi, L., ... Wang, H. (2016). Histone deacetylase inhibitors suppress RSV infection and alleviate virus-induced airway inflammation. *Int J Mol Med*.
- Fenno, J.C. (2005). Laboratory maintenance of *Treponema denticola*. *Curr Protoc Microbiol Chapter 12*, Unit 12B 11.
- Fenno, J. C., Hannam, P. M., Leung, W. K., Tamura, M., Uitto, V. J., & McBride, B. C. (1998). Cytopathic effects of the major surface protein and the chymotrypsinlike protease of *Treponema denticola*. *Infection and Immunity*, 66, 1869–1877.
- Garcia, D., Tarima, S., & Okunseri, C. (2015). Periodontitis and glycemic control in diabetes: NHANES 2009 to 2012. *Journal of Periodontology*, 86, 499–506.

- Ghosh, A., Park, J. Y., Fenno, C., & Kapila, Y. L. (2008). *Porphyromonas gingivalis*, gamma interferon, and a proapoptotic fibronectin matrix form a synergistic trio that induces c-Jun N-terminal kinase 1-mediated nitric oxide generation and cell death. *Infection and Immunity*, 76, 5514–5523.
- Goldberg, A. D., Allis, C. D., & Bernstein, E. (2007). Epigenetics: A landscape takes shape. *Cell*, 128, 635–638.
- Grenier, D., Uitto, V. J., & McBride, B. C. (1990). Cellular location of a *Treponema denticola* chymotrypsinlike protease and importance of the protease in migration through the basement membrane. *Infection and Immunity*, 58, 347–351.
- Guenther, M. G., Levine, S. S., Boyer, L. A., Jaenisch, R., & Young, R. A. (2007). A chromatin landmark and transcription initiation at most promoters in human cells. *Cell*, 130, 77–88.
- Haapasalo, M., Singh, U., McBride, B. C., & Uitto, V. J. (1991). Sulfhydryl-dependent attachment of *Treponema denticola* to laminin and other proteins. *Infection and Immunity*, 59, 4230–4237.
- Hassler, M. R., Klisaroska, A., Kollmann, K., Steiner, I., Bilban, M., Schiefer, A. I., ... Egger, G. (2012). Antineoplastic activity of the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine in anaplastic large cell lymphoma. *Biochimie*, 94, 2297–2307.
- Herceg, Z. (2016). Epigenetic mechanisms as an interface between the environment and genome. *Advances in Experimental Medicine and Biology*, 903, 3–15.
- Hernandez-Barrantes, S., Shimura, Y., Soloway, P. D., Sang, Q. A., & Fridman, R. (2001). Differential roles of TIMP-4 and TIMP-2 in pro-MMP-2 activation by MT1-MMP. *Biochemical and Biophysical Research Communications*, 281, 126–130.
- Hook, K. E., Garza, S. J., Lira, M. E., Ching, K. A., Lee, N. V., Cao, J., ... Pavlicek, A. (2012). An integrated genomic approach to identify predictive biomarkers of response to the aurora kinase inhibitor PF-03814735. *Molecular Cancer Therapeutics*, 11, 710–719.
- Huynh, Q. N., Wang, S., Tafolla, E., Gansky, S. A., Kapila, S., Armitage, G. C., & Kapila, Y. L. (2002). Specific fibronectin fragments as markers of periodontal disease status. *Journal of Periodontology*, 73, 1101–1110.
- Inagaki, S., Kimizuka, R., Kokubu, E., Saito, A., & Ishihara, K. (2016). *Treponema denticola* invasion into human gingival epithelial cells. *Microbial Pathogenesis*, 94, 104–111.
- Jani, J. P., Arcari, J., Bernardo, V., Bhattacharya, S. K., Briere, D., Cohen, B. D., ... Jakubczak, J. L. (2010). PF-03814735, an orally bioavailable small molecule aurora kinase inhibitor for cancer therapy. *Molecular Cancer Therapeutics*, 9, 883–894.
- Jee, S. W., Wang, S., & Kapila, Y. L. (2004). Specific pro-apoptotic fibronectin fragments modulate proteinase expression in periodontal ligament cells. *Journal of Periodontology*, 75, 523–530.
- Jenuwein, T., & Allis, C. D. (2001). Translating the histone code. *Science*, 293, 1074–1080.
- Jo, A. R., Baek, K. J., Shin, J. E., & Choi, Y. (2014). Mechanisms of IL-8 suppression by *Treponema denticola* in gingival epithelial cells. *Immunology and Cell Biology*, 92, 139–147.
- Joo, N. E., Watanabe, T., Chen, C., Chekenya, M., Stallcup, W. B., & Kapila, Y. L. (2008). NG2, a novel proapoptotic receptor, opposes integrin alpha4 to mediate anoikis through PKCalpha-dependent suppression of FAK phosphorylation. *Cell Death and Differentiation*, 15, 899–907.
- Joseph, J., Kapila, Y. L., Hayami, T., & Kapila, S. (2010). Disease-associated extracellular matrix suppresses osteoblastic differentiation of human periodontal ligament cells via MMP-1. *Calcified Tissue International*, 86, 154–162.
- Kapila, Y. L., Kapila, S., & Johnson, P. W. (1996). Fibronectin and fibronectin fragments modulate the expression of proteinases and proteinase inhibitors in human periodontal ligament cells. *Matrix Biology*, 15, 251–261.
- Kapila, Y. L., Lancero, H., & Johnson, P. W. (1998). The response of periodontal ligament cells to fibronectin. *Journal of Periodontology*, 69, 1008–1019.
- Kapila, Y. L., Wang, S., Dazin, P., Tafolla, E., & Mass, M. J. (2002). The heparin-binding domain and V region of fibronectin regulate apoptosis by suppression of p53 and c-myc in human primary cells. *The Journal of Biological Chemistry*, 277, 8482–8491.
- Kapila, Y. L., Wang, S., & Johnson, P. W. (1999). Mutations in the heparin binding domain of fibronectin in cooperation with the V region induce decreases in pp125(FAK) levels plus proteoglycan-mediated apoptosis via caspases. *The Journal of Biological Chemistry*, 274, 30906–30913.
- Khansari, N., Shakiba, Y., & Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation & Allergy Drug Discovery*, 3, 73–80.
- Kim, M. S., Son, M. W., Kim, W. B., In Park, Y., & Moon, A. (2000). Apicidin, an inhibitor of histone deacetylase, prevents H-ras-induced invasive phenotype. *Cancer Letters*, 157, 23–30.
- Kinoshita, T., Sato, H., Okada, A., Ohuchi, E., Imai, K., Okada, Y., & Seiki, M. (1998). TIMP-2 promotes activation of progelatinase A by membrane-type 1 matrix metalloproteinase immobilized on agarose beads. *The Journal of Biological Chemistry*, 273, 16098–16103.
- Koch, C. M., Andrews, R. M., Flicek, P., Dillon, S. C., Karaoz, U., Clelland, G. K., ... Dunham, I. (2007). The landscape of histone modifications across 1% of the human genome in five human cell lines. *Genome Research*, 17, 691–707.
- Konermann, A., Deschner, J., Allam, J. P., Novak, N., Winter, J., Baader, S. L., ... Jager, A. (2012). Antigen-presenting cell marker expression and phagocytotic activity in periodontal ligament cells. *Journal of Oral Pathology & Medicine*, 41, 340–347.
- Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, 128, 693–705.
- Kubicek, S., O'Sullivan, R. J., August, E. M., Hickey, E. R., Zhang, Q., Teodoro, M. L., ... Jenuwein, T. (2007). Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Molecular Cell*, 25, 473–481.
- Lamont, R. J., Chan, A., Belton, C. M., Izutsu, K. T., Vasel, D., & Weinberg, A. (1995). *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infection and Immunity*, 63, 3878–3885.
- Lee, A. Y., Fan, C. C., Chen, Y. A., Cheng, C. W., Sung, Y. J., Hsu, C. P., & Kao, T. Y. (2015). Curcumin inhibits invasiveness and epithelial-mesenchymal transition in oral squamous cell carcinoma through reducing matrix metalloproteinase 2, 9 and modulating p53-E-cadherin pathway. *Integrative Cancer Therapies*, 14, 484–490.
- Leira, Y., Seoane, J., Blanco, M., Rodriguez-Yanez, M., Takkouche, B., Blanco, J. and Castillo, J. (2016). *Association between periodontitis and ischemic stroke: A systematic review and meta-analysis*. *Eur J Epidemiol*.
- Ligi, D., & Mannello, F. (2016). Do matrix metalloproteinases represent reliable circulating biomarkers in colorectal cancer? *British Journal of Cancer*, 115, 633–634.
- Liu, C. C., Chang, T. C., Lin, Y. T., Yu, Y. L., Ko, B. S., Sung, L. Y., & Liou, J. Y. (2016). Paracrine regulation of matrix metalloproteinases contributes to cancer cell invasion by hepatocellular carcinoma-secreted 14-3-3sigma. *Oncotarget*.
- Liu, W. H., Sang, M. X., Hou, S. Y., Zhang, C., & Shan, B. E. (2014). Low-dose decitabine induces MAGE-A expression and inhibits invasion via suppression of NF-kappaB2 and MMP2 in Eca109 cells. *Biomedicine & Pharmacotherapy*, 68, 745–750.
- Liu, Z., Zhang, L., Ding, F., Li, J., Guo, M., Li, W., ... Liu, Z. (2005). 5-Aza-2'-deoxycytidine induces retinoic acid receptor-beta(2) demethylation and growth inhibition in esophageal squamous carcinoma cells. *Cancer Letters*, 230, 271–283.
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔC_t} method. *Methods*, 25, 402–408.
- Lukaszewicz-Zajac, M., Szmitkowski, M., Litman-Zawadzka, A., & Mroczko, B. (2016). Matrix metalloproteinases and their tissue inhibitors in comparison to other inflammatory proteins in gastric cancer (GC). *Cancer Investigation*, 34, 305–312.

- Madsen, D. H., Jurgensen, H. J., Ingvarsen, S., Melander, M. C., Albrechtsen, R., Hald, A., ... Engelholm, L. H. (2013). Differential actions of the endocytic collagen receptor uPARAP/Endo180 and the collagenase MMP-2 in bone homeostasis. *PLoS One*, 8, e71261.
- Makinen, P. L., Makinen, K. K., & Syed, S. A. (1995). Role of the chymotrypsin-like membrane-associated proteinase from *Treponema denticola* ATCC 35405 in inactivation of bioactive peptides. *Infection and Immunity*, 63, 3567–3575.
- Mani, S. K., Kern, C. B., Kimbrough, D., Addy, B., Kasiganesan, H., Rivers, W. T., ... Menick, D. R. (2015). Inhibition of class I histone deacetylase activity represses matrix metalloproteinase-2 and -9 expression and preserves LV function postmyocardial infarction. *American Journal of Physiology. Heart and Circulatory Physiology*, 308, H1391–H1401.
- Martinez, J. A., Milagro, F. I., Claycombe, K. J., & Schalinske, K. L. (2014). Epigenetics in adipose tissue, obesity, weight loss, and diabetes. *Advances in Nutrition*, 5, 71–81.
- Mathers, D. A., Leung, W. K., Fenno, J. C., Hong, Y., & McBride, B. C. (1996). The major surface protein complex of *Treponema denticola* depolarizes and induces ion channels in HeLa cell membranes. *Infection and Immunity*, 64, 2904–2910.
- McDowell, J. V., Frederick, J., Miller, D. P., Goetting-Minesky, M. P., Goodman, H., Fenno, J. C., & Marconi, R. T. (2011). Identification of the primary mechanism of complement evasion by the periodontal pathogen, *Treponema denticola*. *Molecular Oral Microbiology*, 26, 140–149.
- McDowell, J. V., Huang, B., Fenno, J. C., & Marconi, R. T. (2009). Analysis of a unique interaction between the complement regulatory protein factor H and the periodontal pathogen *Treponema denticola*. *Infection and Immunity*, 77, 1417–1425.
- Medina, C., Santana, A., Quintero, E., Radomski, M. W., & Guarner, F. (2004). Matrix metalloproteinases in diseases of the gastrointestinal tract. *Gastroenterología y Hepatología*, 27, 491–497.
- Medzhitov, R., & Horng, T. (2009). Transcriptional control of the inflammatory response. *Nature Reviews. Immunology*, 9, 692–703.
- Miao, D., Fenno, J. C., Timm, J. C., Joo, N. E., & Kapila, Y. L. (2011). The *Treponema denticola* chymotrypsin-like protease dentilisin induces matrix metalloproteinase-2-dependent fibronectin fragmentation in periodontal ligament cells. *Infection and Immunity*, 79, 806–811.
- Miao, D., Godovikova, V., Qian, X., Seshadrinathan, S., Kapila, Y. L., & Fenno, J. C. (2014). *Treponema denticola* upregulates MMP-2 activation in periodontal ligament cells: Interplay between epigenetics and periodontal infection. *Archives of Oral Biology*, 59, 1056–1064.
- Mirza, S., Sharma, G., Pandya, P., & Ralhan, R. (2010). Demethylating agent 5-aza-2-deoxycytidine enhances susceptibility of breast cancer cells to anticancer agents. *Molecular and Cellular Biochemistry*, 342, 101–109.
- Mittal, R., Patel, A. P., Debs, L. H., Nguyen, D., Patel, K., Grati, M., ... Liu, X. Z. (2016). Intricate functions of matrix metalloproteinases in physiological and pathological conditions. *Journal of Cellular Physiology*, 231, 2599–2621.
- Mogal, A., & Abdulkadir, S. A. (2006). Effects of histone deacetylase inhibitor (HDACi); Trichostatin-A (TSA) on the expression of housekeeping genes. *Molecular and Cellular Probes*, 20, 81–86.
- Mosig, R. A., & Martignetti, J. A. (2013). Loss of MMP-2 in murine osteoblasts upregulates osteopontin and bone sialoprotein expression in a circuit regulating bone homeostasis. *Disease Models & Mechanisms*, 6, 397–403.
- Murphy, G., Stanton, H., Cowell, S., Butler, G., Knauper, V., Atkinson, S., & Gavrilovic, J. (1999). Mechanisms for pro matrix metalloproteinase activation. *APMIS*, 107, 38–44.
- Nagaraju, G. P., Aliya, S., Zafar, S. F., Basha, R., Diaz, R., & El-Rayes, B. F. (2012). The impact of curcumin on breast cancer. *Integr Biol (Camb)*, 4, 996–1007.
- Nagase, H. (1997). Activation mechanisms of matrix metalloproteinases. *Biological Chemistry*, 378, 151–160.
- Nagase, H., Visse, R., & Murphy, G. (2006). Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research*, 69, 562–573.
- Nagasri, M., Madhulatha, M., Musalaih, S. V., Kumar, P. A., Krishna, C. H., & Kumar, P. M. (2015). Efficacy of curcumin as an adjunct to scaling and root planning in chronic periodontitis patients: A clinical and microbiological study. *Journal of Pharmacy & Bioallied Sciences*, 7, S554–S558.
- Nakayama, J., Rice, J. C., Strahl, B. D., Allis, C. D., & Grewal, S. I. (2001). Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science*, 292, 110–113.
- Navratilova, Z., Kolek, V., & Petrek, M. (2016). Matrix metalloproteinases and their inhibitors in chronic obstructive pulmonary disease. *Archivum Immunologiae et Therapiae Experimentalis (Warsz)*, 64, 177–193.
- Nebbioso, A., Carafa, V., Benedetti, R., & Altucci, L. (2012). Trials with 'epigenetic' drugs: An update. *Molecular Oncology*, 6, 657–682.
- Niller, H. H., Masa, R., Venkei, A., Meszaros, S., & Minarovits, J. (2017). Pathogenic mechanisms of intracellular bacteria. *Current Opinion in Infectious Diseases*, 30, 309–315.
- Nissinen, L., & Kahari, V. M. (2014). Matrix metalloproteinases in inflammation. *Biochimica et Biophysica Acta*, 1840, 2571–2580.
- Nowakowska, A., & Tarasiuk, J. (2016). Comparative effects of selected plant polyphenols, gallic acid and epigallocatechin gallate, on matrix metalloproteinases activity in multidrug resistant MCF7/DOX breast cancer cells. *Acta Biochimica Polonica*.
- Offenbacher, S., Barros, S. P., & Beck, J. D. (2008). Rethinking periodontal inflammation. *Journal of Periodontology*, 79, 1577–1584.
- Oyarzun, A., Arancibia, R., Hidalgo, R., Penafiel, C., Caceres, M., Gonzalez, M. J., ... Smith, P. C. (2010). Involvement of MT1-MMP and TIMP-2 in human periodontal disease. *Oral Diseases*, 16, 388–395.
- Pardo, A., Cabrera, S., Maldonado, M., & Selman, M. (2016). Role of matrix metalloproteinases in the pathogenesis of idiopathic pulmonary fibrosis. *Respiratory Research*, 17, 23.
- Park, S. Y., Jun, J. A., Jeong, K. J., Heo, H. J., Sohn, J. S., Lee, H. Y., ... Kang, J. (2011). Histone deacetylases 1, 6 and 8 are critical for invasion in breast cancer. *Oncology Reports*, 25, 1677–1681.
- Pasquier, J., Hoarau-Vechot, J., Fakhro, K., Rafii, A., & Abi Khalil, C. (2015). Epigenetics and cardiovascular disease in diabetes. *Current Diabetes Reports*, 15, 108.
- Pereira, I. T., Ramos, E. A., Costa, E. T., Camargo, A. A., Manica, G. C., Klassen, L. M., ... Klassen, G. (2014). Fibronectin affects transient MMP2 gene expression through DNA demethylation changes in non-invasive breast cancer cell lines. *PLoS One*, 9, e105806.
- Perkins, D. J., Patel, M. C., Blanco, J. C., & Vogel, S. N. (2016). Epigenetic mechanisms governing innate inflammatory responses. *Journal of Interferon & Cytokine Research*, 36, 454–461.
- Pietruszewska, W., Bojanowska-Pozniak, K., & Kobos, J. (2016). Matrix metalloproteinases MMP1, MMP2, MMP9 and their tissue inhibitors TIMP1, TIMP2, TIMP3 in head and neck cancer: An immunohistochemical study. *Otolaryngologia Polska*, 70, 32–43.
- Pihlstrom, B. L., Michalowicz, B. S., & Johnson, N. W. (2005). Periodontal diseases. *The Lancet*, 366, 1809–1820.
- Prasanna, S. J. (2011). Causal relationship between periodontitis and chronic obstructive pulmonary disease. *J Indian Soc Periodontol*, 15, 359–365.
- Razzouk, S., & Sarkis, R. (2013). Smoking and diabetes. Epigenetics involvement in osseointegration. *The New York State Dental Journal*, 79, 27–30.
- Safronova, O., & Morita, I. (2010). Transcriptome remodeling in hypoxic inflammation. *Journal of Dental Research*, 89, 430–444.
- Sakamoto, M., Takeuchi, Y., Umeda, M., Ishikawa, I., & Benno, Y. (2001). Rapid detection and quantification of five periodontopathic bacteria by real-time PCR. *Microbiology and Immunology*, 45, 39–44.
- Saldanha, S. N., Kala, R., & Tollefsbol, T. O. (2014). Molecular mechanisms for inhibition of colon cancer cells by combined epigenetic-modulating epigallocatechin gallate and sodium butyrate. *Experimental Cell Research*, 324, 40–53.

- Scanlon, C., Marchesan, J., Soehren, S., Matsuo, M., & Kapila, Y. (2011). Capturing the regenerative potential of periodontal ligament fibroblasts. *J Stem Cells Regen Med*, 7, 54–56.
- Schoffski, P., Jones, S. F., Dumez, H., Infante, J. R., Van Mieghem, E., Fowst, C., ... Burris, H. A. (2011). Phase I, open-label, multicentre, dose-escalation, pharmacokinetic and pharmacodynamic trial of the oral aurora kinase inhibitor PF-03814735 in advanced solid tumours. *European Journal of Cancer*, 47, 2256–2264.
- Shin, J., & Choi, Y. (2012). The fate of *Treponema denticola* within human gingival epithelial cells. *Molecular Oral Microbiology*, 27, 471–482.
- Shinkarenko, T. V., Rumiantsev, V. A., Egorova, E. N., & Eliseeva, T. I. (2013). Matrix metalloproteinases in periodontitis. *Stomatologiya (Mosk)*, 92, 77–80.
- Shofuda, K., Moriyama, K., Nishihashi, A., Higashi, S., Mizushima, H., Yasumitsu, H., ... Miyazaki, K. (1998). Role of tissue inhibitor of metalloproteinases-2 (TIMP-2) in regulation of pro-gelatinase activation catalyzed by membrane-type matrix metalloproteinase-1 (MT1-MMP) in human cancer cells. *Journal of Biochemistry*, 124, 462–470.
- Simonson, L. G., Goodman, C. H., Bial, J. J., & Morton, H. E. (1988). Quantitative relationship of *Treponema denticola* to severity of periodontal disease. *Infection and Immunity*, 56, 726–728.
- Sitholmelma, C. S., & Shangase, L. S. (2013). The association between periodontitis and pre-term birth and/or low birth weight: A literature review. *SADJ*, 68, 162–166.
- Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent, R. L. Jr. (1998). Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology*, 25, 134–144.
- Song, S. E., Choi, B. K., Kim, S. N., Yoo, Y. J., Kim, M. M., Park, S. K., ... Kim, C. K. (2003). Inhibitory effect of procyanidin oligomer from elm cortex on the matrix metalloproteinases and proteases of periodontopathogens. *Journal of Periodontal Research*, 38, 282–289.
- Stewart, R., & West, M. (2016). Increasing evidence for an association between periodontitis and cardiovascular disease. *Circulation*, 133, 549–551.
- Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A., & Goldberg, G. I. (1995). Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. *The Journal of Biological Chemistry*, 270, 5331–5338.
- Tafolla, E., Wang, S., Wong, B., Leong, J., & Kapila, Y. L. (2005). JNK1 and JNK2 oppositely regulate p53 in signaling linked to apoptosis triggered by an altered fibronectin matrix: JNK links FAK and p53. *The Journal of Biological Chemistry*, 280, 19992–19999.
- Talbert, P. B., & Henikoff, S. (2006). Spreading of silent chromatin: Inaction at a distance. *Nature Reviews. Genetics*, 7, 793–803.
- Tanaka, K., Iwasaki, K., Feghali, K. E., Komaki, M., Ishikawa, I., & Izumi, Y. (2011). Comparison of characteristics of periodontal ligament cells obtained from outgrowth and enzyme-digested culture methods. *Archives of Oral Biology*, 56, 380–388.
- Uitto, V. J., Grenier, D., Chan, E. C., & McBride, B. C. (1988). Isolation of a chymotrypsinlike enzyme from *Treponema denticola*. *Infection and Immunity*, 56, 2717–2722.
- Veeraraghavan, J., Natarajan, M., Lagisetty, P., Awasthi, V., Herman, T. S., & Aravindan, N. (2011). Impact of curcumin, raspberry extract, and neem leaf extract on rel protein-regulated cell death/radiosensitization in pancreatic cancer cells. *Pancreas*, 40, 1107–1119.
- Waddington, C. H. (2012). The epigenotype. 1942. *International Journal of Epidemiology*, 41, 10–13.
- Wang, F., Qi, Y., Li, X., He, W., Fan, Q. X., & Zong, H. (2013). HDAC inhibitor trichostatin A suppresses esophageal squamous cell carcinoma metastasis through HADC2 reduced MMP-2/9. *Clinical and Investigative Medicine*, 36, E87–E94.
- Williams, R. C., Barnett, A. H., Claffey, N., Davis, M., Gadsby, R., Kellett, M., ... Thackray, S. (2008). The potential impact of periodontal disease on general health: A consensus view. *Current Medical Research and Opinion*, 24, 1635–1643.
- Yin, L., & Chung, W. O. (2011). Epigenetic regulation of human beta-defensin 2 and CC chemokine ligand 20 expression in gingival epithelial cells in response to oral bacteria. *Mucosal Immunology*, 4, 409–419.
- Yoshida, A., Kawada, M., Suzuki, N., Nakano, Y., Oho, T., Saito, T., & Yamashita, Y. (2004). TaqMan real-time polymerase chain reaction assay for the correlation of *Treponema denticola* numbers with the severity of periodontal disease. *Oral Microbiology and Immunology*, 19, 196–200.
- Zhang, M., Xiao, X. Q., Jiang, Y. F., Liang, Y. S., Peng, M. Y., Xu, Y., & Gong, G. Z. (2011). DNA demethylation in PD-1 gene promoter induced by 5-azacytidine activates PD-1 expression on Molt-4 cells. *Cellular Immunology*, 271, 450–454.
- Zhang, S., Barros, S. P., Niculescu, M. D., Moretti, A. J., Preisser, J. S., & Offenbacher, S. (2010). Alteration of PTGS2 promoter methylation in chronic periodontitis. *Journal of Dental Research*, 89, 133–137.
- Zhang, Y., & Reinberg, D. (2001). Transcription regulation by histone methylation: Interplay between different covalent modifications of the core histone tails. *Genes & Development*, 15, 2343–2360.
- Zucker, S., Pei, D., Cao, J., & Lopez-Otin, C. (2003). Membrane type-matrix metalloproteinases (MT-MMP). *Current Topics in Developmental Biology*, 54, 1–74.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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