

Review

Epigenetics and Its Role in Periodontal Diseases: A State-of-the-Art Review

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The immune response to oral bacteria and the subsequent activation of inflammatory signaling is not only dependent on genetic factors. The importance of so-called epigenetic mechanisms presents additional regulatory pathways of genes involved in maintaining chronic inflammation, including gingivitis and periodontitis. The term *epigenetics* relates to changes in gene expression that are not encoded in the DNA sequence itself and include chemical alterations of DNA and its associated proteins. These changes lead to remodeling of the chromatin and subsequent activation or inactivation of a gene. Epigenetic mechanisms have been found to contribute to disease, including cancer and autoimmune or inflammatory diseases. In this state-of-the-art review, the authors provide the latest findings on the involvement of epigenetic modifications in the development of periodontal disease and present emerging therapeutic strategies aimed at epigenetic targets (epidrugs) associated with the disruption of tissue homeostasis and the development of periodontitis. *J Periodontol* 2015;86:556-568.

KEY WORDS

Acetylation; DNA methylation; epigenomics; genetics; histones; periodontitis.

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Periodontitis is an infectious disease characterized by chronic inflammation in the gingival tissues in response to bacteria colonizing the tooth surface, ultimately leading to tissue breakdown with loss of alveolar bone and connective tissue.¹ The epithelial cells in the oral cavity are constantly being exposed to high levels of various bacteria, which leads to activation of multiple signaling pathways that induce various inflammatory response mechanisms.¹ The host inflammatory response to bacteria is in turn influenced by both genetic and environmental factors.^{1,2}

REGULATION OF IMMUNE RESPONSE

The central dogma of gene expression is that DNA is transcribed into messenger RNA (mRNA), which serves as a template for the protein synthesis.³ The basic model for the transcriptional induction in the immune response is that external stimuli, such as bacterial products, start a signaling cascade within the cells, activating the binding of transcription factors to regulatory sequences of the DNA, so-called promoters and enhancers. This assembles the transcription initiation machinery and induces the expression of certain gene sets.⁴ At this level, genetic changes such as mutations, polymorphisms, or alterations in the DNA sequence alter the binding sites for transcription factors in the DNA regulatory region (promoter), thereby affecting

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not only the initiation speed but also the magnitude of the gene expression. In addition, the form of the protein, should these changes occur within the coding regions of the gene (exons), results in changes in the amino acid sequence of the protein.⁵

A recent study has revealed additional regulatory layers superimposed on this basic model.⁴ Specific functional programs able to be rapidly turned on and off in response to external stimuli have been identified.^{4,6} These processes have been proposed as a mechanism in creating a selectivity of the host response to stimuli, and also to regulate the magnitude of the inflammatory response to clear the

pathogens or drive chronic inflammation.⁷ In addition, this presents a potential mechanism of how different bacterial species lead to activation of signaling pathways, inducing inflammatory response mechanisms.⁸

The layers of gene expression regulation of an immune response include structures of the chromatin, transcription factors, and posttranscriptional modifications, e.g., RNA splicing, mRNA polyadenylation, and regulatory molecules, which include microRNA (miRNA) and the recently identified long non-coding RNAs (lncRNAs)⁹ (Fig. 1). The first two mechanisms are: 1) modifications of the mRNA transcript and 2) regulation of the stability, storage, and amount of mRNA transcripts present in the cytoplasm. When DNA is transcribed into mRNA, a process called splicing removes the non-coding sequences of mRNA (introns). Bacterial products can alter the splicing pattern, resulting in different isoforms of the protein produced. After the transcription and splicing, the mRNA transcript acquires a sequence of adenosine bases at the terminal end of the transcript called a poly (A) tail. The length of this poly(A) tail determines and contributes to both mRNA stability and transport to the cytoplasm. It also acts as a mechanism for the cell to store silenced mRNA within the cytoplasm, which in response to bacterial stimuli can be used to rapidly induce gene expression without having to start the DNA transcription process in the nucleus. This was also suggested as a way to limit immune response to avoid inducing chronic inflammation.¹⁰

Not all RNA is protein coding. miRNAs are small, evolutionarily conserved ncRNAs that are derived from longer transcripts encoded within the genome. They inhibit gene expression by binding to mRNA by a sequence-pairing homology to regulate and fine-tune gene expression. miRNA expression is altered in response to bacterial products, but inflammatory cytokines can also influence the regulation of

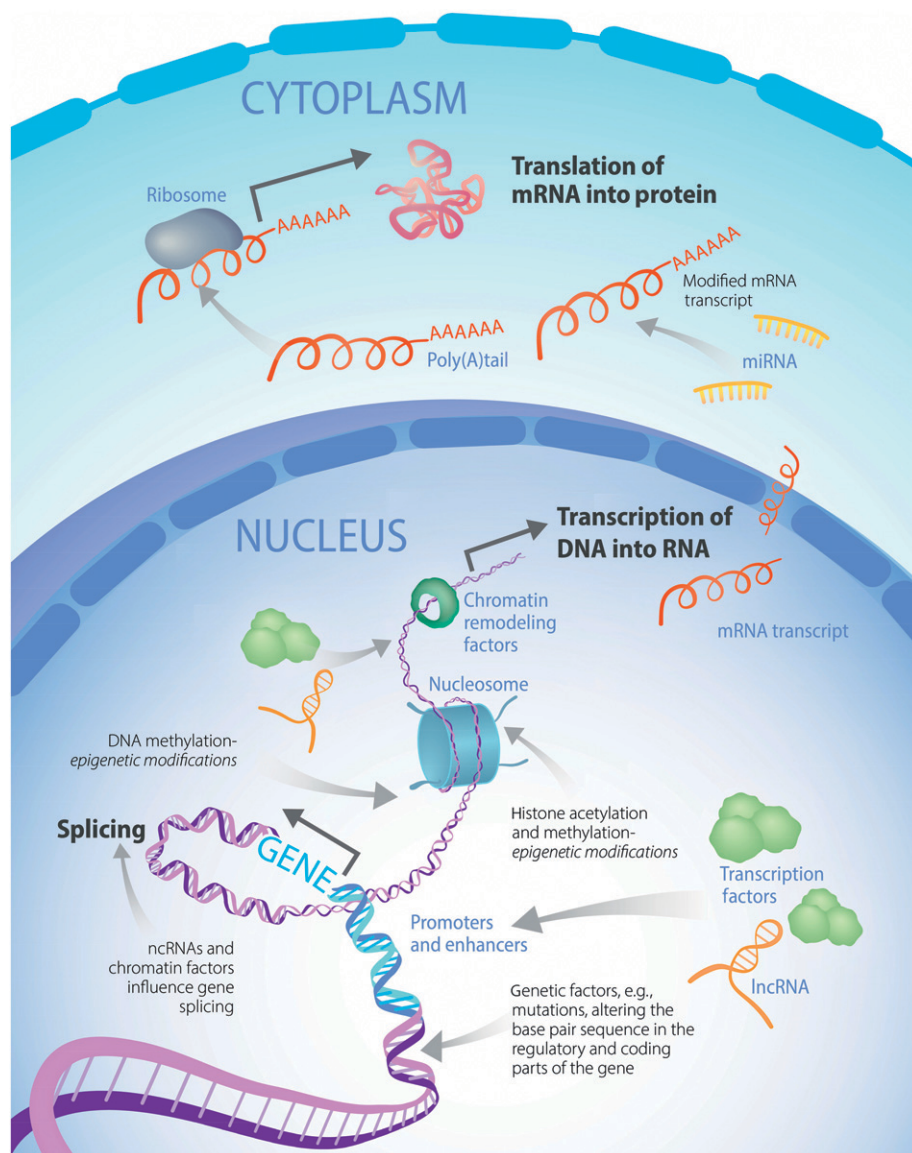


Figure 1.

Epigenetic studies have identified multiple layers of regulatory factors and mechanisms that add to the basic model for transcriptional induction in the immune response. Bacterial products and the inflammatory process influence the transcriptional cascade at all levels.

miRNA production.⁷ Recently, another class of ncRNAs was discovered, the lncRNAs.⁷ Different biologic functions have been attributed to lncRNAs. A potential mechanism for their ability to regulate gene expression is that they bind to chromatin regulatory proteins, thereby controlling access or inhibition of proteins binding to enhancer regions in the DNA.^{3,11,12} One function of these RNAs in the immune response has been suggested to involve the regulation of the host response, including innate immunity, since virus infection alters the expression of lncRNAs.¹³ Interestingly, a recent report identified a new ncRNA that is linked to epigenetic mechanisms.¹⁴

Chronic inflammatory diseases, such as periodontitis, have specific target tissues in which the inflammation results in tissue breakdown.¹ A specific gene may possess different epigenetic patterns de-

pending on the cell type, resulting in local and systemic expression of the gene. This indicates a local, site-specific change in the immune response to external stimuli and/or the inflammatory response itself, which may differ among individuals and add to the susceptibility to disease. This review focuses on the epigenetic alterations of the chromatin and DNA, how they can contribute to periodontal disease, and the recent literature on their potential use as new treatment targets.

EPIGENETICS

The term *epigenetics* relates to changes in gene expression that are not encoded in the DNA sequence.¹⁵ Epigenetic modifications include chemical alterations of DNA and associated proteins, leading to remodeling of the chromatin and activation or inactivation of a gene. These changes can contribute

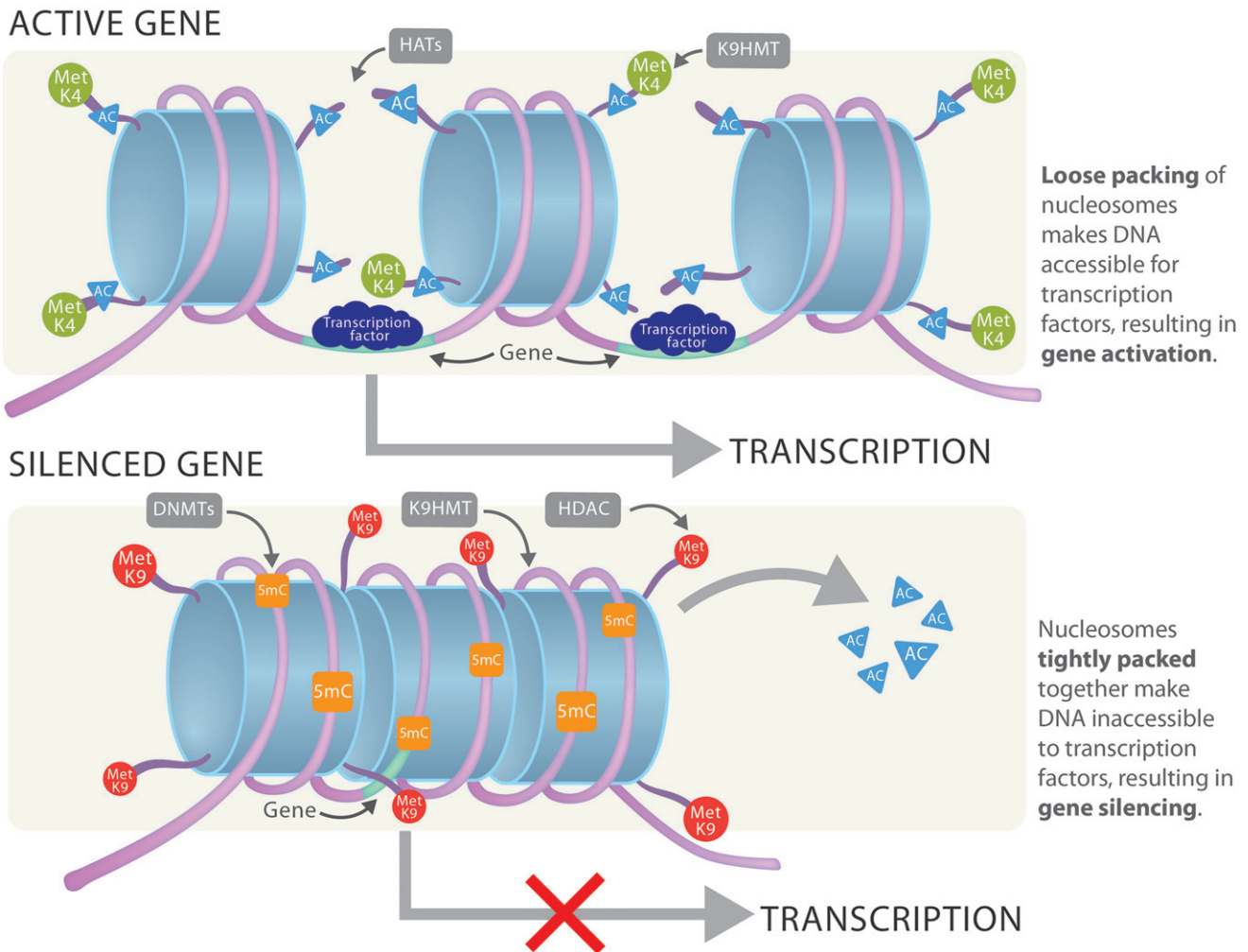


Figure 2.

Schematic illustration of the influence of histone acetylation on gene transcription. Met = methylated; DNMTs = DNA methyltransferases; 5mC = 5-methylcytosine, K9HMT = Lysine 9 histone methyltransferase; AC = acetylation.

to the development and maintenance of cancer and autoimmune or inflammatory diseases, including periodontitis. Interestingly, some epigenetic modifications are reversible and can be induced and/or altered by environmental factors, therefore presenting a link between the inherited genome and the environment.^{16,17} The two major epigenetic modifications are DNA methylation and histone acetylation and methylation.¹⁷

The building blocks of chromatin are the nucleosomes.¹⁷ One nucleosome consists of 146 base pair (bp) DNA and a core histone complex, which includes two copies each of histones H2A, H2B, H3, and H4 and the linker histone H1 that connects the nucleosomes, forming the primary chromatin structure, often referred to as “beads-on-a-string.” Histones can be acetylated or methylated at histone amino acid tails that protrude from the nucleosome.^{18,19} Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs).²⁰ Removal of acetyl groups by HDACs leads to alterations in the packing of DNA around histones and a subsequent inactivation of genes (Fig. 2). In contrast, hyperacetylation is associated with transcriptionally active chromatin.^{20,21} Methylation occurs at different lysine molecules on histones H3 and H4, with H3 lysines K4 and K9 being most commonly methylated.¹⁹ Although high levels of methylated K4 have been associated with transcriptionally active regions, methylated K9 was found in silent chro-

matin regions.¹⁹ Importantly, a lysine can be mono-, di-, or tri-methylated, further adding to the various functions of methylated histones.¹⁹ However, recent research in the area of histone methylation reveals evidence for an even more complicated regulation and function of histone methylation, with specific methylation patterns in promoters, introns, and exons resulting in a fine-tuning of gene expression.²²

In the classic DNA methylation model, the addition of methyl groups to cytosine bases situated next to a guanine base occurs at specific sites in the DNA sequence, so-called CpG islands or CpG sites by the de novo DNA methyltransferases (DNMTs) DNMT3a and DNMT3b.^{15,23} This results in both methylated as well as unmethylated CpG sites in the DNA strands. The maintenance enzyme DNMT1 then remethylates hemimethylated CpG sites.²⁴ This modification alters the configuration of the DNA and the binding of transcription factors, resulting in changes in gene expression. Transcriptionally active genes are associated with low levels of DNA methylation. However, in 2014, Jeltsch and Jurkowska²⁴ presented a modified version of this classic model based on recent research findings. It was suggested that the average DNA methylation (global DNA methylation) level of DNA is inherited rather than the methylation state of individual CpG sites (site-specific DNA methylation). To add another level to the concept of DNA methylation, it was discovered that 5-methylcytosine (5mC) could be further oxidized into 5-hydroxymethylcytosine (5hmC) by the ten-eleven translocation (TET) family of enzymes.²⁵ The TET enzymes can then convert 5hmC into unmethylated cytosine, resulting in DNA hypomethylation.²⁶ The biologic function of 5hmC is not clear, but it has been suggested to be an intermediate leading to demethylation of 5mC and thereby re-expression of genes silenced by DNA methylation (Fig. 3).²⁷

Histone modifications and DNA methylation are not separate events but are linked, and the resulting combinations promote unique cellular responses.^{21,23} In addition, hypo-acetylated histones and hypermethylated CpGs in chronic inflammatory diseases may result in persistent inflammation.²⁰ In a recent study, treatment of mice with a compound capable of inducing an immune response (antigen stimulation) resulted in a decrease of DNMT1 followed by an increase in HDAC3.²⁸

These changes may provide evidence of the individual differences in local expression of genes associated with inflammation. Specific factors have been reported as risk factors for periodontitis, e.g., smoking, age, bacteria, and genetic polymorphisms. Association between age and risk of developing periodontitis has been reported.^{29,30} Comparison of epigenetic patterns over time in twins at different

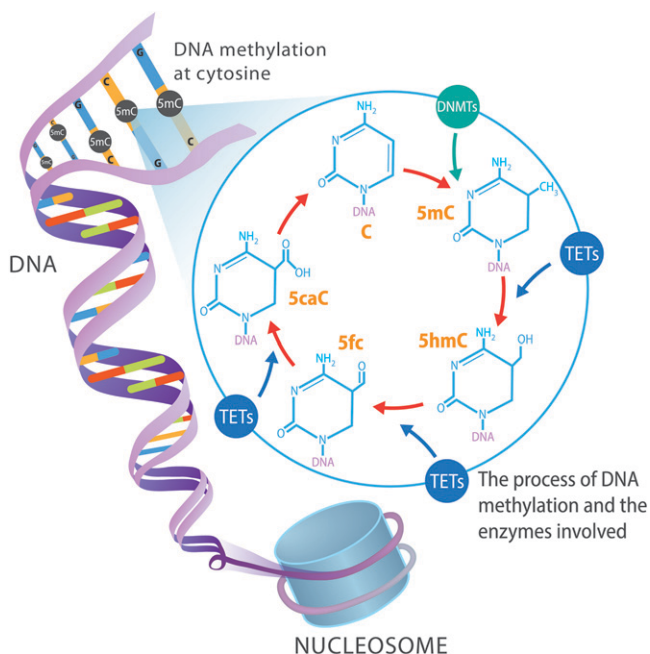


Figure 3.

Illustration of the different forms of DNA methylation and the proteins regulating this process. 5caC = 5-carboxydeoxyctidine, 5fc = 5-formyldeoxyctidine, c = cytosine.

Table 1.
Overview of Studies on Epigenetic Mechanisms in Relation to Periodontitis

Study	Mechanism	Marker	Material	Method	Results
Andia et al. 2010 ³⁵	DNA methylation	IL-8	Epithelial cells from 37 patients with generalized AgP and 37 controls	MSP	The IL-8 promoter was found to be hypomethylated in individuals with generalized AgP.
Baptista et al. 2014 ⁴⁴	DNA methylation	SOCS1, LINE-1	Oral cells from 30 patients with generalized AgP and 30 healthy controls	COBRA	A higher percentage of demethylation of SOCS1 was found for the healthy group. A similar result was reported for the LINE-1 gene.
Barros and Offenbacher 2014 ³³	DNA methylation	Genome-wide screening	Gingival tissue samples from healthy sites and diseased sites from patients with periodontitis and tissue samples from healthy individuals	Microarray, LCM	Several thousand genes were analyzed, and differences in DNA methylation were found between healthy and inflamed tissue.
Cantley et al. 2011 ⁴⁶	Histone analysis	Periodontal bone loss	Experimental periodontitis mouse model	Microcomputed tomography, histologic analysis	HDACi was reported to suppress bone loss. Differences were found for the different HDACis used.
de Faria Amorimino et al. 2013 ⁴³	DNA methylation	TLR2	Gingival biopsies from 20 patients with periodontitis and 20 controls	Methyl profiler DNA methylation qPCR assay	Hypermethylation of TLR2 was reported for the periodontitis group.
de Oliveira et al. 2011 ⁴²	DNA methylation	TLR2, TLR4	Gingival tissue from 11 controls, 11 smokers with periodontitis, and 12 non-smokers with periodontitis	Methylation analysis using specific methylation-sensitive restriction enzymes and PCR	TLR4 was unmethylated in most samples in all groups, whereas the TLR2 gene was found to be a mix of both methylated and unmethylated sites in all groups.
Ishida et al. 2012 ³⁷	DNA methylation	IL-6	Peripheral blood samples from 30 patients with CP, 30 patients with RA, and 30 controls	Bisulfite genomic sequencing	The methylation level of the CpG motif -74 bp was lower in patients with RA and CP compared with controls.

Table 1. (continued)
Overview of Studies on Epigenetic Mechanisms in Relation to Periodontitis

Study	Mechanism	Marker	Material	Method	Results
Larsson et al. 2012 ⁴⁰	DNA methylation, histone analysis	IL-10	Gingival biopsies and peripheral blood samples from six individuals with periodontitis; lymphoblastoid cell lines	Bisulfate sequencing, HRM, ChIP	LPS stimulation of B cells resulted in an increase in acetylation of H4 and methylation of H3. Treatment with HDACi and 5-aza resulted in increase in IL-10 mRNA expression. The three CpG sites in the distal IL-10 promoter region were methylated in both blood and gingival tissue.
Loo et al. 2010 ⁴⁵	DNA methylation	E-Cadherin, COX-2	Blood samples from 108 periodontally healthy controls, 110 patients with CP, and 106 patients with breast cancer	MSP	Hypermethylation of E-Cadherin and COX-2 was reported for the CP group and the cancer group. None was found in the healthy group.
Oliveira et al. 2009 ³⁴	DNA methylation	IL-18	Epithelial cells from 41 controls, 30 smokers with CP, and 40 non-smokers with CP; blood leukocytes and gingival cells from 13 individuals from each group	MSP	Inflammation in the oral mucosa influenced the DNA methylation pattern of IL-8. No differences were found in methylation between blood leukocytes and gingival cells. A higher percentage of methylation frequency was found in controls compared with patients with periodontitis, in which most samples had a high level of unmethylation.
Stefani et al. 2013 ³⁶	DNA methylation	IL-6	Gingival biopsies from 21 patients with CP and 21 controls	MSP	No difference in methylation level of IL-6 was found within the regions analyzed.
Viana et al. 2011 ³⁸	DNA methylation	IFN- γ , IL-10	Gingival biopsies from 18 patients with periodontitis and 16 controls	MSP, bisulfite sequencing	The methylation level of IFN- γ and IL-10 was similar in both groups. Most samples were positive for IFN- γ methylation. Of samples in the periodontitis group, 11% were unmethylated, whereas no unmethylated samples were found for IL-10.

Table 1. (continued)
Overview of Studies on Epigenetic Mechanisms in Relation to Periodontitis

Study	Mechanism	Marker	Material	Method	Results
Zhang et al. 2010 ³⁹	DNA methylation	IFN- γ	Gingival biopsies from 23 periodontally healthy sites, 12 experimentally induced gingivitis sites, and 12 CP sites	Pyrosequencing	An overall lower DNA methylation level was found in the periodontitis group compared with periodontally healthy and gingivitis groups.
Zhang et al. 2010 ⁴⁷	DNA methylation	PTGS2	10 biopsies from inflamed gingival tissue and six non-inflamed gingival tissue biopsies	Bisulfite-specific PCR, cloning, and sequencing	In inflamed gingival tissue, hypermethylation of the PTGS2 promoter was associated with lower levels of PTGS2 transcription.
Zhang et al. 2013 ⁴¹	DNA methylation	TNF- α	Gingival biopsies from 17 patients with CP, 18 periodontally healthy individuals, and 11 with experimental gingivitis; THP1 and RAW264.7 cell lines	Bisulfite-specific PCR and pyrosequencing	Two CpG sites in the TNF- α promoter had a higher level of DNA methylation in biopsies from patients with periodontitis compared with those from control patients. Treatment with 5-aza resulted in an increase in TNF- α mRNA expression.

IL = interleukin; AgP = aggressive periodontitis; MSP = methylation-specific polymerase chain reaction; SOCS1 = suppressor of cytokine signaling 1; LINE-1 = long interspersed element-1; COBRA = combined bisulfite restriction analysis; LCM = laser capture microdissection; HDACi = histone deacetylase inhibitor; TLR2 = Toll-like receptor 2; qPCR = quantitative polymerase chain reaction; CP = chronic periodontitis; RA = rheumatoid arthritis; HRM = high-resolution melting curve analysis; ChIP = chromatin immunoprecipitation assay; 5-aza = 5-aza-deoxycytidine; COX-2 = cyclooxygenase 2; IFN = interferon; PTGS2 = prostaglandin-endoperoxide synthase 2; TNF- α = tumor necrosis factor- α .

ages showed large differences in gene expression patterns, indicating that epigenetic changes increase during life. However, age itself contrasts with the genetic changes seen in twins, since age itself is a risk factor for epigenetic changes.³¹ In addition, it has been suggested that the epigenome of older patients with periodontitis may differ from that of younger patients.³² Epigenetic mechanisms may also explain why some patients may not respond to treatment and why some individuals are more susceptible to periodontitis.³² Studies on epigenetic mechanisms in relation to periodontitis³³⁻⁴⁷ are presented in Table 1.

DNA Methylation and Periodontitis

In periodontitis, epigenetic modifications during inflammation occur locally at the biofilm-gingival interface around the teeth. It has been reported that the epigenome differs between inflamed periodontal sites and non-inflamed sites in the same individual.³³ In addition, different methylation patterns associated with pathways regulating cell differentiation, apoptosis, lipopolysaccharide (LPS)-mediated signaling, oncogenesis, and cell adhesion were found in inflamed tissue from patients with periodontitis compared with tissue from healthy individuals.³³

Several studies have investigated the DNA methylation of inflammatory cytokines in chronic periodontitis (CP) and aggressive periodontitis (AgP).³⁴⁻⁴¹ Analysis of the interleukin (IL)-8 promoter indicates a tissue-specific pattern in DNA methylation.³⁴ In epithelial cells, the methylation frequency of IL-8 was higher in controls than in patients with periodontitis. No difference was seen in gingival cells or blood leukocytes.³⁴ These findings are in line with data that found that the IL-8 promoter was hypomethylated in oral epithelial cells from individuals with generalized AgP compared with healthy controls.³⁵ The IL-6 promoter was found to be partially methylated in both healthy individuals and patients with periodontitis,³⁶ even though the level of expression of IL-6 was higher in patients with periodontitis. A similar

increase in IL-6 serum level in patients with CP compared with healthy individuals was associated with hypomethylation of the -74 bp CpG site in the IL-6 promoter. This suggests that DNA methylation of a single CpG site may influence cytokine production and hence disease.³⁷

The methylation level of interferon (IFN)- γ has been investigated in two studies.^{38,39} Viana et al.³⁸ reported a similar methylation level of IFN- γ and IL-10 in gingival biopsies from patients with CP compared with biopsies from healthy controls. In contrast, Zhang et al.³⁹ reported a lower methylation level of CpG sites in periodontitis biopsies compared with periodontally healthy tissue biopsies. This was related to an increase in IFN- γ transcription. The increase in IFN- γ in experimental gingivitis was found to be independent of promoter methylation. Regarding the *IL-10* gene, no unmethylated samples were found in either periodontitis or healthy tissues.³⁸ Three CpG sites close to the IL-10 promoter region around the -1,087 polymorphism were methylated in DNA from peripheral blood cells and from gingival tissue. Treatment of B cells with the DNA methylation inhibitor 5-aza-deoxycytidine resulted in an increase in IL-10 mRNA regardless of genotype for the -1,087 polymorphism.⁴⁰ Two CpG sites in the tumor necrosis factor (TNF)- α promoter were reported to be hypermethylated in CP. In addition, transcription of TNF- α was shown to be regulated by the methylation level, since treatment of ThP1 cells with 5-aza-deoxycytidine increased TNF- α expression.⁴¹

In addition to methylation level of cytokines, a number of other genes related to inflammation have been analyzed in periodontitis.⁴²⁻⁴⁵ The DNA methylation pattern in the Toll-like receptor 2 (*TLR2*) and *TLR4* genes was evaluated in gingival tissue samples.⁴² The *TLR4* gene promoter was unmethylated in most samples in all groups,⁴² whereas the *TLR2* promoter was both methylated and unmethylated in most samples. A hypermethylation and a subsequent low transcription of *TLR2* in periodontitis tissues was reported.⁴³ Interestingly, a positive correlation was found between *TLR2* methylation and periodontal probing depth. In a study on AgP, an overall demethylation pattern of the suppressor of cytokine signaling 1 (*SOCS1*) gene was found.⁴⁴ In healthy individuals, this demethylated level was higher, and also total demethylated samples were found for this group compared to periodontitis. A significant difference was also reported regarding the methylation pattern for the long interspersed element-1 (*LINE-1*) gene.⁴⁴

The methylation status of the E-Cadherin and cyclooxygenase 2 (*COX-2*) genes was investigated in patients with CP and patients with breast cancer.⁴⁵ The results showed a hypermethylation of these two genes in both patients with periodontitis and those

with cancer compared with healthy individuals. These findings confirm the present view that chronic inflammation and cancer may have a similar epigenetic pattern and that DNA methylation may be a link between inflammation and cancer.⁴⁸

A mechanism of how DNA methylation of a gene promoter may influence gene expression was illustrated in a study by Zhang et al.⁴⁷ in which hypermethylation of the prostaglandin-endoperoxide synthase 2 (*PTGS2*) promoter in inflamed periodontal tissue was associated with lower levels of *PTGS2* transcription. Interestingly, a CpG site close to a nuclear factor (NF)- κ B transcription factor-binding site was highly methylated, suggesting that methylation status may exclude binding of this transcription factor. This is in line with other studies on methylation of cytokine promoter regions in which the CpG sites analyzed were in close proximity to binding sites for transcription factors NF- κ B and specificity protein 1 (Sp1).^{40,41} These two factors are known to be strong regulators of gene expression of the immune response. They are activated by bacteria binding to TLRs and the subsequent activation of mitogen-activated protein kinase (MAPK) pathways.^{49,50} The influence of Sp1 and NF- κ B on the chromatin structure was demonstrated by Widlak et al. in 1997,⁵¹ who showed that the binding of Sp1 and NF- κ B to adjacent sites induced a repositioning of the nucleosomes upstream and downstream of the factors, thereby influencing gene transcription.⁵¹ It has been reported that enzymes that cause methylation can be regulated directly or indirectly by the extracellular milieu through signal transduction pathways such as the MAPK extracellular signal-regulated kinase (ERK) kinase pathway.⁵²

The inflammatory processes may also result in aberrant DNA methylation in tissues.⁵³ This mechanism has been suggested as a link between inflammation and alterations in methylation level in cancers. In inflamed tissue, neutrophils and eosinophils produce substances that further form halogenated cytosine products.⁵³ The DNMT1 enzyme cannot distinguish between halogenated and methylated DNA, resulting in an increase in de novo methylation in cells of the inflamed tissue.⁵³ In addition, antigen stimulation can result in downregulation of DNMT1 and HDAC2, with an increase in HDAC3, together with induction of Sp1 and NF- κ B p65 binding to the HDAC3 promoter. The downregulation of DNMT1 results in induction of adhesion molecules and cellular interactions.²⁸

The process of 5mC oxidation into 5hmC has been well studied in cancer, but understanding of the role of these markers in inflammation is limited. In a study on the autoimmune disease systemic lupus erythematosus (SLE),⁵⁴ the levels of TET1, TET2, and 5hmC in cluster of differentiation 4-positive (CD4⁺) T cells were

compared to those from healthy individuals. A high level of these markers in patients with SLE was found. It was suggested that the DNA hydroxymethylation may be contributing to the pathogenesis of SLE.⁵⁴ Because SLE and periodontitis share common features regarding the immune response, it may also be that DNA hydroxymethylation plays a role in periodontitis similar to that in SLE.⁵⁵ Bacterial influence of TET2 expression has been previously shown, as *Escherichia coli* stimulation of THP1 cells for 24 hours resulted in more than double the amount of TET2 mRNA.⁵⁶

Histone Modifications and Periodontitis

Few studies are available on histone modifications in periodontitis.^{40,46} Maintaining histone acetylation of genes related to osteoclastogenesis was found to be important for preventing bone loss in experimental periodontitis. Treatment with histone deacetylase inhibitor (HDACi) resulted in improved bone levels.⁵³ In a study from the authors' laboratory,⁴⁰ it was demonstrated that different epigenetic modifications of DNA and histones influence *IL-10* gene expression. The authors also found an association between LPS stimulation and histone modifications. Furthermore, these alterations differed among *IL-10* genotypes for the -1,087 G/A polymorphism in the *IL-10* gene promoter.⁴⁰ Administration of HDACi trichostatin A did not alter the *IL-10* production; however, treatment using HDACi butyrate and valproic acid resulted in an increase in *IL-10* gene expression.

Influence of Periodontal Pathogens on Chromatin Modifications

An early report on the bacterial influence on DNA methylation showed that bacterial infection with *Campylobacter rectus* promoted DNA hypermethylation of the insulin-like growth factor 2 (*IGF2*) gene.⁵⁷ Stimulation with the periodontal pathogen *Treponema denticola* on periodontal ligament cells showed a decrease in methylation of the matrix metalloproteinase (MMP)-2 promoter.⁵⁸ However, the authors could not confirm these findings using the more sensitive bisulfite DNA sequencing method.⁵⁸ In addition, the MMP-2 promoter was found to be hypomethylated regardless of the presence of *T. denticola*. It was suggested that the adherence and/or internalization of *T. denticola* may contribute to regulation of matrix degradation and bone resorption.⁵⁸ Studies have shown that the culture supernatant from the periodontal pathogen *Porphyromonas gingivalis* contains high levels of butyric acid.^{59,60} This culture supernatant was reported to inhibit HDACs, resulting in increased histone acetylation.^{59,60}

Periodontal pathogens can induce cell-specific changes as shown in a study⁶¹ comparing the expression of epigenetic markers in gingival fibroblasts and keratinocytes. *P. gingivalis* LPS stimulation re-

sulted in a decrease in mRNA transcription of DNMT1, DNMT3, and histone demethylases Jumonji domain containing 3 (*JMJD3*) in keratinocytes but not in gingival fibroblasts. In contrast, RNA analysis from gingival biopsies from periodontitis and healthy sites showed no difference in gene expression for these target genes.⁶¹ *JMJD3* binds to genes and regulates the H3K27me3 level and hence transcription activity. It was suggested that a decrease in *JMJD3* could lead to changes in transcription levels of inflammatory genes activated by LPS. Interestingly, *JMJD3* is regulated by NF- κ B.⁶¹ Consistent with these findings are the results presented by Yin and Chung,⁸ who investigated the influence of *P. gingivalis* and *Fusobacterium nucleatum* on epigenetic modifications in gingival epithelial cells. *P. gingivalis* stimulation resulted in a decrease in HDAC1, HDAC2, and DNMT1, whereas no difference was found after stimulation with *F. nucleatum* for those markers compared with untreated cells. These results indicate that oral bacterial infection is associated with changes in the histone H3 lysine 4 (H3K4) methylation level, and that the bacteria investigated induced different levels of trimethylated H3K4. In addition, these bacteria drove different epigenetic changes for the antimicrobial proteins human β -defensin 2 and CC chemokine ligand 20.

Specific cells within the oral mucosa may respond differently to bacterial infection or to the periodontal inflammatory processes.³⁴ This results in differences in the methylation pattern of a certain gene among cell types. Analysis of epigenetic modifications from gingival biopsies shows the combined results of all cells present in the sample, making it difficult to estimate the role of each cell type in the epigenetic pattern at the site of inflammation.⁶¹ Barros and Offenbacher used laser capture microdissection (LCM) to analyze epithelial cells from the sulcular epithelium.³³ A large number of genes were analyzed, but the gamma-amino-n-butyrate transaminase (*GATA*) gene better illustrated differences in gene expression between the gingival biopsies and LCM tissue samples. The results from gingival samples showed a 2.5-fold increase in *GATA* gene methylation, comparing healthy individuals versus patients with periodontitis. This was confirmed using LCM, with which a 1.2-fold increase was found.

However, not only do bacteria influence the methylation level of a host's genes, the bacterial DNA can also differ in methylation level, which influences their ability to induce the production of inflammatory cytokines.⁶² The production of TNF- α in macrophages was induced only when bacterial DNA from *Aggregatibacter actinomycetemcomitans*, *P. gingivalis*, *Peptostreptococcus micros*, and *E. coli* was unmethylated.⁶²

The studies on epigenetics and periodontitis describe how the immune response influences the epigenetic pattern of genes. However, it should be noted that epigenetic changes may also affect the immune response itself, thereby providing a potential explanation for how individuals are more prone to develop chronic inflammation.

CLINICAL APPLICATIONS TARGETING EPIGENETIC MODIFICATIONS

Limited research has been done using HDAC or DNA methylation inhibitors as treatments for periodontitis or oral inflammation. In contrast, there are numerous reports on the use of these epigenetic inhibitors in cancer research. In a recent review,⁶³ different areas for use of epigenetic factors were identified to improve individualized drug therapy, in which pharmacogenetic biomarkers can be used to predict drug response. One example is the O6-methylguanine DNA methyltransferase molecule that can be detected in serum and saliva at levels corresponding to that of the primary tumor. In this manner, it would be possible to monitor epigenetic changes in a specific tissue by using more easily accessible body fluids. Epidrugs are defined as “drugs that inhibit or activate disease-associated epigenetic proteins for ameliorating, curing, or preventing the disease.”⁶³ Indeed, the present authors have found that administration of epidrugs (HDACi) can prevent the accumulation of

head and neck cancer stem cells and increase the sensitivity of tumor cells to chemotherapy.^{64,65} At present, two DNMT inhibitors and two HDAC inhibitors have been approved by the U.S. Food and Drug Administration. Although these developments are exciting, the complexity of solid tumors are far beyond current understanding, and the influence of gene expression mediated by histone modifications is in part impacted by the latest identification of several mutations in histones and histone modifiers.⁶⁶

Recently, HDACis have been found not only to regulate histone acetylation but also to influence DNA methylation.⁵² The HDACi suberoylanilide hydroxamic acid inhibits histone acetylation but also regulates DNMT1 protein expression.⁵² Treatment using another HDAC inhibitor, 1,179.4b (Classes I and II HDACi), showed reduced alveolar bone loss and less osteoclasts per square millimeter in mice with *P. gingivalis*-induced periodontitis compared with untreated mice.⁴⁶ However, no influence was found on the level of inflammation, which was suggested to indicate a direct effect of this HDACi on bone regeneration rather than an indirect effect by targeting the inflammation.⁴⁶ Moderate daily exercise and diet were found to reduce *P. gingivalis*-induced periodontitis in diet-induced obese mice by restoring the level of histone 3 acetylation to normal, as well as NF- κ B recruitment to TNF- α and IL-10 promoter.⁶⁷ It should be noted that *P. gingivalis*

produces butyric acid that inhibits HDACs and increases histone acetylation, which was shown to reactivate Epstein-Barr virus (EBV) as well as human immunodeficiency virus 1, indicating that periodontal disease may contribute to EBV-related diseases.^{59,60}

A group of epigenetic regulatory proteins are the bromodomain and extraterminal domain (BET) proteins, which are able to read acetylated histone tails and recruit transcription complexes to regulate gene transcription. In a recent study, the BET inhibitor JQ1 was found to inhibit both an inflammatory response and alveolar bone loss in experimental periodontitis. In vitro analysis of the function of JQ1 revealed that binding of the BET protein BRD4 to the NF- κ B promoters was decreased, as was NF- κ B phosphorylation, and treatment with JQ1 also attenuated nuclear NF- κ B. Based on these findings, it

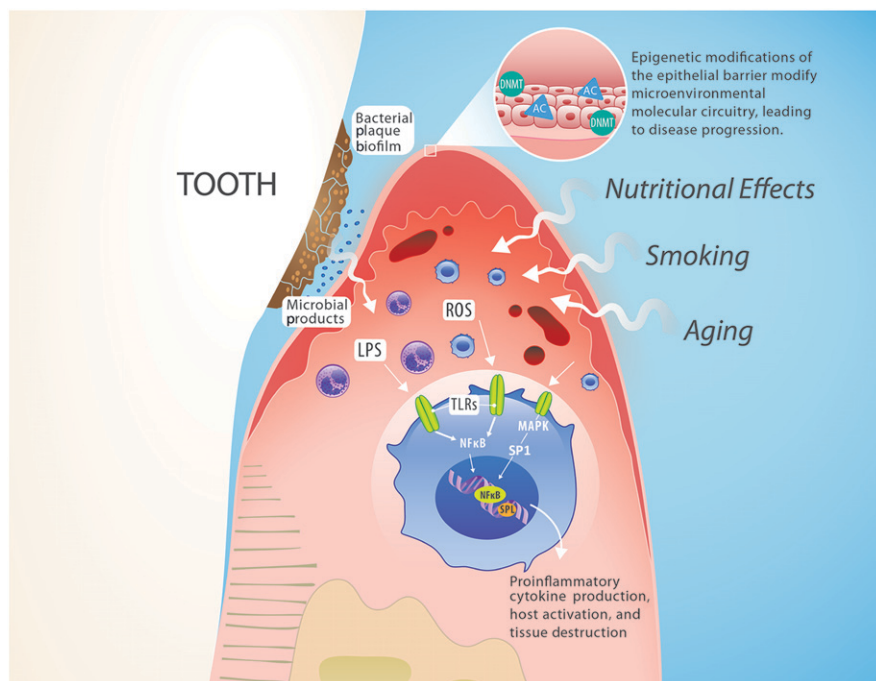


Figure 4.

Based on the studies presented in the present review, a potential hypothesis of the epigenetic events occurring in a CP lesion. ROS = reactive oxygen species; AC = acetylase; SPI = transcription factor.

was suggested that JQ1 may be a potential treatment model for periodontitis.⁶⁸ Interestingly, aspirin has been found to have a negative effect on allergic inflammation by regulating DNMT1 expression. In this model, aspirin was shown to prevent antigen from influencing the expression of HAT Tip60, HDAC3, and DNMT1.²⁸

CONCLUSIONS

Recent studies have reported on the ability and importance of environmental factors to induce changes in epigenetic regulatory mechanisms of the immune response (Fig. 4). Knowledge of the role of epigenetics in development of periodontal diseases is still limited, but the studies presented in this review highlight that both DNA methylation and histone modifications occur in the oral mucosa in response to bacteria and the inflammatory processes. To better understand the influence of environmental cues during the development of periodontal disease, the molecular events driven by epigenetic modifications need to be identified. This information may also provide some explanations as to why patients with similar clinical characteristics respond differently to treatment. Identifying epigenetic patterns associated with the development of periodontitis may also improve personalized approaches that can be used to individualize treatment plans for patients with periodontitis.

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