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Epigenetics in susceptibility, progression, and diagnosis of periodontitis

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ABSTRACT

Periodontitis is characterized by irreversible destruction of periodontal tissue. At present, the accepted etiology of periodontitis is based on a three-factor theory including pathogenic bacteria, host factors, and acquired factors. Periodontitis development usually takes a decade or longer and is therefore called chronic periodontitis (CP). To search for genetic factors associated with CP, several genome-wide association study (GWAS) analyses were conducted; however, polymorphisms associated with CP have not been identified. Epigenetics, on the other hand, involves acquired transcriptional regulatory mechanisms due to reversibly altered chromatin accessibility. Epigenetic status is a condition specific to each tissue and cell, mostly determined by the responses of host cells to stimulations by local factors, like bacterial inflammation, and systemic factors such as nutrition status, metabolic diseases, and health conditions. Significantly, epigenetic status has been linked with the onset and progression of several acquired diseases. Thus, epigenetic factors in periodontal tissues are attractive targets for periodontitis diagnosis and treatments. In this review, we introduce accumulating evidence to reveal the epigenetic background effects related to periodontitis caused by genetic factors, systemic diseases, and local environmental factors, such as smoking, and clarify the underlying mechanisms by which epigenetic alteration influences the susceptibility of periodontitis.

Contents

Abbreviations: CP, chronic periodontitis; GWAS, genome-wide association study; H3K9ac, histone H3 lysine 9; H3K27ac, acetylation of histone H3 lysine 27; H3K4me3, trimethylation of histone H3 lysine 4; H3K27me3, trimethylation of histone H3 lysine 27; PDL, periodontal ligament; AP, aggressive periodontitis; ncRNAs, non-coding RNAs; lncRNAs, long ncRNAs; ECM, extracellular matrix; HDACs, histone deacetylases; ceRNA, competing endogenous RNA; LPS, lipopolysaccharide; HATs, histone acetyltransferases; m^6A, N_6-methyladenosine; sEV, small extracellular vesicles; 5mC, 5-methylcytocine; ATAC-seq, assay for transposase-accessible chromatin sequencing

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1. Introduction

Epigenetic status involves the reversibly altered chromatin accessibility brought about by chemical modifications of histone, DNA, and higher-order chromatin structure, and regulates transcriptional events without changing the genomic DNA sequence [1] (Fig. 1). The epigenetically modified chromatin structure is called the epigenome. Local active chromatin exhibits a stretched chromatin structure with active marks such as acetylation of histone H3 lysine 9 (H3K9ac), acetylation of histone H3 lysine 27 (H3K27ac), trimethylation of histone H3 lysine 4 (H3K4me3), and demethylated DNA. The stretched chromatin structure is convenient for transcriptional complexes to associate local genomic DNA and transcript RNAs. Conversely, local suppressive chromatin exhibits a compressed chromatin structure with suppressive marks, such as trimethylation of histone H3 lysine 27 (H3K27me3) and methylated DNA, and the compressed chromatin structure prevents binding of transcriptional complexes to genomic DNA [1]. Epigenetic status is presumed to be constructed by reflecting on various environmental cues [2]. The epigenetic statuses of each tissue and cell are generated due to additive or synergistic responses to various local and systemic factors [1,3]. Several in vitro studies demonstrated that most epigenetic patterns were passed to daughter cells after cell division and the epigenetic status would be fixed [4]. The existence of epigenetic memory in vivo has been recently disclosed [5]. PPARα is a key transcriptional factor of hepatic lipid metabolism by regulating transcription of the genes coding for the proteins working in energy metabolism pathways [5–8]. Mice received a synthetic PPARα ligand during the perinatal period through maternal administration, which resulted in diminished diet-induced obesity in adulthood. Moreover, PPARα administration induced demethylation of the *FGF-21* gene locus in the postnatal period and demethylation status persisted in adulthood. Since demethylation of the *FGF-21* gene locus increased *FGF-21* transcription, *FGF-21* secretion from the liver occurred to ameliorate adiposity in the epididymal white adipose tissue [5]. Therefore, epigenetic status is potentially more stable than previously thought.

Periodontitis is a common disease accompanied by irreversible destruction of periodontal tissue such as alveolar bone, cementum, periodontal ligament (PDL), and gingival tissue. The disease is developed by local pathogenic bacterial infections and involves the host's protective inflammatory reactions, which release cytokines and enzymes that induce the breakdown of periodontal connective tissue and bone [9]. Periodontitis is pathophysiologically diverse because various acquired local factors, such as oral hygiene, odontoparallaxis, and occlusal trauma, and systemic factors, such as nutrition, metabolic diseases, and diabetes, intricately influence the status of the disease. Development of periodontitis usually takes a decade or longer, so the age of onset is usually over 40, and this major type of periodontitis is called chronic periodontitis (CP). A small portion of periodontitis patients possesses severe phenotypes from prepubertal or adolescent age, and this type of periodontitis is called aggressive periodontitis (AP) or early-onset periodontitis; however, CP and AP phenotypes are currently characterized by the same indicators, using a multi-dimensional staging and grading system [10,11]. Periodontal diseases also develop as one of the manifestations of systemic genetic disorders, such as immunologic disorders, which include Papillon-Lefèvre syndrome, Chediak-Higashi syndrome, Cohen syndrome, and Ehlers-Danlos syndrome, and bone metabolism disorders such as hypophosphatasia [12]. These clinical observations suggest that genetic and epigenetic factors have predominant roles for the development of periodontitis. In this review, we introduce accumulating evidence to reveal the involvement of genetic and epigenetic backgrounds on CP and AP, and we introduce mechanistic analyses to clarify underlying mechanisms by which epigenetic alteration influences susceptibility of periodontitis.

2. Genetic factors of periodontitis

2.1. Genetic factors of CP

Studies on the *IL-1* gene cluster associated with CP found that the *IL-1β* (3953/4) C/T polymorphism (rs1143634) was positively associated with the severity of CP in Caucasians [13], and a meta-analysis of case-control studies concluded that the association was not significant in Asians [14]. Thus far, several genome-wide association study (GWAS) analyses of CP have been conducted, and none of the single-nucleotide polymorphisms that meet the criteria of significant threshold ($P < 5 \times 10^{-8}$) were identified [15–19]. Single nucleotide polymorphisms in gene loci of *KCNQ5* and *GPR141-NME8* were identified as potential loci for clinical periodontitis in a Japanese population [15]. Single nucleotide polymorphisms in gene loci of *NIN*, *NPY*, and *WNT5a* were identified as potential loci for severe CP, and gene loci of *NCR2* and *EMR1*, and the genomic region on chromosome 10p15 were identified as potential loci for moderate CP [16]. A GWAS report identified potential single nucleotide polymorphisms in gene loci of *LAMA2*, *HAS2*, *CDH2*, and *ESR1*, and in the genomic regions on chromosome 14q21–22 between *SOS2* and *NIN*, chromosome 3q22 near *OSBPL10*, chromosome 4p15 near *HSP90AB2P*, chromosome 11p15 near *GVINP1*, chromosome 14q31 near *SEL1L*, and chromosome 18q12 in *FHOD3* [17]. No genome-wide significant gene loci in CP, regardless of severity, race, gender, or age, indicates that genetic background is not influential on CP, and potential effects of genetic background may be dominated by multiple environmental factors.

2.2. Genetic factors of AP

In contrast to CP, single nucleotide polymorphisms in gene loci of *GLT6D1*, *SIGLEC5*, and *DEFA1A3* were significantly enriched in AP patients investigated by GWAS analyses of German and Dutch case-control samples [20–22]. GATA-3 potentially associates to the gene loci of *GLT6D1*, and a rare mutation identified in AP patients resulted in decreased binding ability of GATA-3, examined by in vitro study [21]. Thus, GATA-3 mediated signaling is possibly a key factor for developing AP. GWAS analysis of AP patients in a Japanese population identified putative polymorphism in the *GPR126* gene locus, and in vitro analysis implied that this polymorphism influenced the functions of *GPR126* for osteogenic differentiation of PDL cells [22,23]. Furthermore, 2 studies conducting whole exome sequencing of AP families and sporadic patients from different groups identified 8 mutation points in *NOD2* [24,25]. Among these, p.Arg311Trp mutations were commonly identified in both studies [24,25]. *NOD2* is an intracellular sensor of bacterial peptidoglycan and participates in innate immunity. The polymorphisms in *NOD2* were linked with increased risk of Crohn's disease, which is an inflammatory bowel disease [26,27]. *NOD2* possesses 3 functional domains including caspase activation and recruitment domains, nucleotide-binding domains, and leucine-rich repeat domains. Significantly, the mutations associated with AP were detected in all three domains [24]. So far, functional alteration of *NOD2* caused by each mutation has not been revealed. Most recently, exosome sequencing of children who developed stage IV, grade C periodontitis identified mutations in gene loci of *CTSC*, *TUT7*, *PADI1*, *FLG*, *ABCA1*, *GLT6D1*, and *SIGLEC5* [28]. Thus, the genetic background responsible for AP has mostly been clarified.

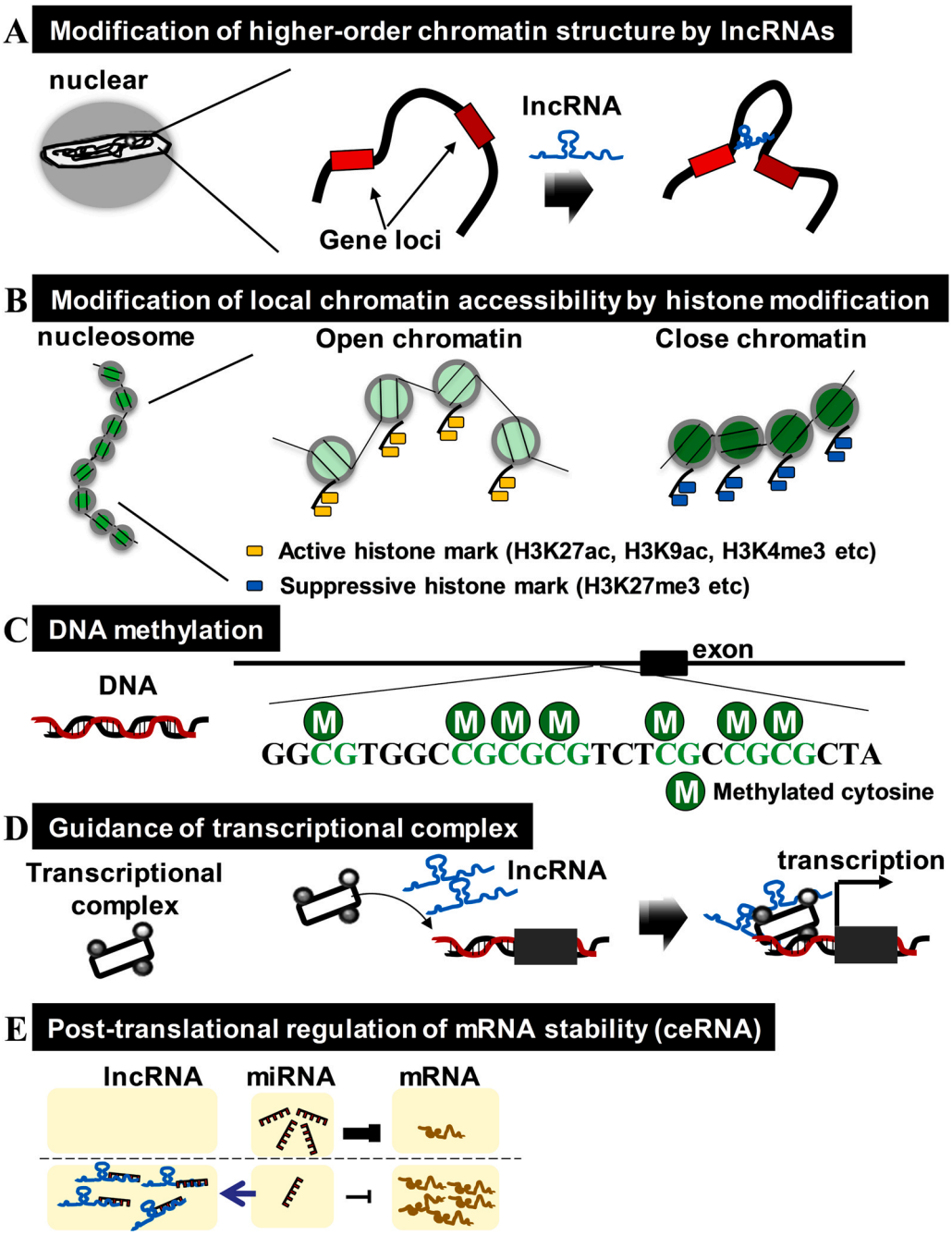

Fig. 1. Epigenetic regulation of gene expression, (A) Modification of higher-order chromatin structure by lncRNAs. (B) Modification of local chromatin accessibility by histone modification. Accessible chromatin is marked with active chromatin marks such as H3K27ac, H3K9ac, and H3K4me3. Inaccessible chromatin is marked with suppressive chromatin marks such as H3K27me3. (C) DNA methylation. Cytosine methylation suppresses gene expression. (D) Guidance of transcriptional complex. ncRNAs guide the transcriptional complex to the promoter region to induce gene expression. (E) Post-translational regulation of mRNA stability (ceRNA). lncRNAs bind to miRNA to inhibit miRNA-induced mRNA degradation.

3. Epigenetic factors of periodontitis

3.1. Studies focusing on DNA methylation status in pre-selected gene loci

Methylated DNA constructs compressed chromatin structure, and the degree of local DNA methylation, particularly in CpG islands, is inversely correlated with gene expression. Before high-throughput techniques became accessible, characteristic DNA methylation changes in CP were investigated by looking in pre-selected genomic positions. Methylation of six CpG sites in *IFNγ* promoter was significantly lower, and the *IFNγ* expression level was significantly higher (1.96-fold) in periodontitis gingival biopsy samples compared with healthy biopsy samples [29] (Table 1). The differences seemed to originate from infiltration of $CD4^+$ T cells, $CD11C^+$ cells, and $CD56^+$ NK cells expressing *IFNγ* in periodontitis tissues. Similarly, comparison of DNA methylation between CP and healthy gingival biopsy samples showed hyper-methylation of the *TLR2* gene locus and a low expression of the *TLR2* gene [30], no difference in methylation pattern of the *IL6* promoter despite increased *IL6* expression in periodontitis samples [31], and the methylation level of the *TNFα* promoter, at −163 bp, was significantly increased despite higher *TNFα* expression in periodontitis samples [32]. The pilot case-control study to evaluate the changes of DNA methylation in inflammatory

Table 1
Identification of epigenetic periodontitis markers in clinical samples.

Study	Published year	Analysis	Assay type	Target	Subjects	Samples	Finding
Zhang et al.[28]	2010	DNA methylation	PCR	*IFNγ* promoter	47	gingival tissue	Decreased methylation and increased transcription of *IFNγ* in periotonditis biopsies ($p = 0.007$)
de Faria Amormino et al.[29]	2013	DNA methylation	PCR	*TLR2* promoter	40	gingival tissue	Hypermethylation and low transcription of *TLR2* in CP bioposies ($p < 0.001$)
Stefani et al.[30]	2013	DNA methylation	PCR	SNP − 174 G/C in the *IL6* promoter	42	gingival tissue	No difference of methylation pattern between control and CP
Zhang et al.[31]	2013	DNA methylation	PCR	*TNFα* promoter region	46	gingival tissue	Decreased methylation at − 163 and − 161 bp and increased transcription of *TNFα* in CP
Asa'ad et al.[32]	2017	DNA methylation	PCR	*LINE1*, *COX2*, *IFNγ*, *TNFα* gene loci	20	gingival tissue	Decreased methyaltion level of *COX2* after periodontal treatment ($p < 0.05$).
Shaddox et al.[33]	2017	DNA methylation	Array	Inflammatory candidate genes	25	gingival tissue	Decreased CpG methylation level of CCL25 and IL17C in AP
De Souza et al.[34]	2014	DNA methylation	Array	immune-related, cell cycle-related, and stablye-expressed gene loci	23	gingival tissue	Decreased methylation status of immune-related gene loci in CP
Azevedo et al.[36]	2020	DNA methylation	Array	T-cell and B-cell function regulators, transcriptional and translational regulators, and cytokine pathway-related genes	40	gingival tissue	Hypomethylation of *STAT5* in periodontitis samples
Kim et al.[37]	2021	DNA methylation	Array	whole-genome	36	gingival tissue	Total 43 631 defferentially methylated postions and 12 genes linked with differentatial methylation in stage III periodontitis
Hernández et al.[38]	2021	DNA methylation	Array	whole-genome	18	peripheral leukocytes	Hypermethylation of *ZNF718* and *HOXA4* and hypomethylated of *ZFP57* in periodontitis
Asa'ad et al.[50]	2020	miRNA	Meta-analysis	Selected 16 miRNAs	–	gingival tissue	Dominant expression of miRNA-142–3p and miRNA-146a in periodontitis
Micó-Martínez et al.[51]	2021	miRNA	Meta-analysis	miRNAs expressed in periodontal tissue	–	gingival tissue	Impossiblity of conducting meta-analysis due to methodological differences among the selected literatures

gene loci before and after the periodontal treatment of CP revealed that *COX2* gene loci were hypo-methylated after the periodontal treatment. However, methylation of *LINE-1*, *IFNγ*, and *TNFα* gene loci was sustained [33]. Studies focusing on AP patients showed that CpG methylation in *CCL25* and *IL17C* gene loci was significantly suppressed in the gingival tissue of AP patients compared with healthy control samples by methylation detection PCR array comprised of 22 inflammatory candidate genes [34].

3.2. Studies conducting high-throughput analyses

High-throughput DNA analysis focusing on genomic positions ranging from − 2000 to + 2000 bp from the transcriptional start site showed that DNA methylation of the genes related to immune processes and inflammatory responses was lower in periodontitis gingival samples compared with healthy gingival samples, and the trend was negatively correlated with previously published gene expression array data [35,36] (Table 1). These results suggested that epigenetic modulation by DNA methylation impacted the prognosis of periodontitis. DNA methylation detection panels comprised of T-cell and B-cell function regulators, transcriptional and translational regulators, and cytokine pathway-related genes found that hypo-methylation of the *STAT5* gene locus occurred in gingival samples of CP, in contrast with that of healthy control samples [37]. More recently, combination analysis of mRNA transcription by RNA-seq of poly-A-tailed RNAs and differential DNA methylation by a genome wide-coverage DNA methylation microarray panel, including the spots of CpG islands, gene bodies, and enhancers, found that 43 631 and 536 differentially methylated positions occurred in periodontitis and gingivitis samples, respectively, compared with healthy samples [38]. These data suggested drastic epigenetic changes in periodontitis gingival tissues. Among the neighboring genes of differentially methylated positions in periodontitis samples, 11 genes, including *KCNA3*, *KCNA2*, *RIMS2*, *HOXB7*, *PNOC*, *IRX1*, *JSRP1*, *TBX1*, *OPCML*, *CECR1*, and *SCN4B,* were differentially expressed. Genome-wide DNA methylation analysis of peripheral leukocytes revealed that hyper-methylation in *ZNF718* and *HOXA4* gene loci and hypo-methylation in the *ZFP57* gene locus were differentially identified in periodontitis patients compared with healthy controls [39]. These results indicated that systemic and local epigenetic patterns seem to be independent, and the differences were at least partially brought about by the cell type because gingival samples contain heterogeneous cell populations. Particularly, immune cell proportions in inflamed gingival tissue, non-inflamed gingival tissue adjacent to periodontitis region, and healthy gingival tissue were 52%, 28%, and 16%, respectively [40]. Higher proportions of TET2-positive cells were observed in the periodontitis lesion, which indicated that a higher demethylation activity might also be responsible for identifying different outcomes for the epigenetic panel between peripheral leukocytes and periodontitis gingival tissue [41].

3.3. Current research analyzing epigenetics and epigenetic modulators in periodontitis other than DNA methylation

Epigenetics encompasses DNA methylation, histone methylation, histone acetylation, and modification of non-coding RNAs (ncRNAs), including short RNAs, such as miRNAs and long ncRNAs (lncRNAs) (Fig. 1). As previously discussed, most clinical studies investigating the involvement of epigenetic action for periodontitis have focused on DNA methylation, presumably because it's easier to prepare many samples for high-throughput analysis [42–45]. One most recent study revealed that H3K27me3, a suppressive mark, was strongly enriched, and that enrichment of H3K27me3 was closer to transcriptional start sites in periodontitis samples compared with healthy control samples, which implied closed chromatin construction in extracellular matrix (ECM)-related gene loci [46]. The oral pathogens dysregulated chromatin-modifying enzymes such as histone deacetylases (HDACs) by changing their expression level and induced whole genomic alteration of histone modifications [47]. Mechanistic analysis revealed that specific inhibition of HDAC3 efficiently reduced inflammatory responses in *Prophyromonas gingibalis* (*P.g.*) stimulated gingival fibroblasts [48]. ncRNAs are defined as molecules without apparent protein-coding potential that regulate complex cellular behaviors, and they are classified into small RNAs such as miRNAs and lncRNAs by length [49,50]. Meta-analysis of miRNA expression in periodontitis and peri-implantitis concluded that miRNA-146a and miRNA-142–3p were statistically significant in periodontitis patients [51], and miRNA-146a is one of the well-analyzed miRNAs in periodontitis [52]. Notably, miR-146a was reported as negatively regulating the innate immune system, and the concentrations were significantly higher in CP patients, and miRNA-146a expression was inversely correlated with *TNFα* and *IL6* expression [53]. lncRNAs participate in various aspects of cellular events such as modification of higher-order chromatin structure and transactivation of transcriptional factor complex [54,55]. In addition, lncRNAs is known to act as a competing endogenous RNA (ceRNA) by directly binding to target miRNAs. As miRNA captured by lncRNA loses its ability to degrade miRNA-targeted mRNA, lncRNA expression facilitates miRNA-targeted mRNA stabilization and protein synthesis [56]. Thus, regarding the regulatory network (i.e., lncRNAs-miRNAs-mRNAs) for periodontitis, lncRNAs (e.g., *MALAT1*, *TUG1*, *FGD5-AS1*, *LINC00687*, *LBX-AS1*, *LINC01566*) were listed as key molecules to bridge genetic (e.g., mRNA) and epigenetic (e.g., miRNA and lncRNA) mechanisms for ceRNA networks [57,58].

4. Epigenetic association between periodontitis and systemic diseases/conditions

Periodontitis is deeply linked with systemic chronic inflammatory diseases and conditions such as nutritional status, metabolic diseases, obesity, and diabetes mellitus [59–62]. The epigenetic status of periodontal tissues is influenced by the local environment and systemic conditions. Whole-genome DNA methylation analyses of gingival tissue from minipigs with streptozotocin-evoked experimental diabetes revealed distinct patterns of hyper- and hypo-methylation compared with minipigs that didn't have diabetes [63]. Since hypo-methylation was significantly identified in pro-inflammatory gene loci, such as *TNFα* and *IL6*, the systemic diabetes condition may epigenetically promote inflammation in physiologically normal gingival tissue and increase the susceptibility of periodontitis. Investigation of the clinical relationship between periodontitis and systemic diseases has been conducted by analyzing genomic DNA methylation of peripheral blood. By focusing on CpG motifs in the *TNFα* gene locus, 1 motif in CP patients and 7 motifs in rheumatoid arthritis patients were identified as having significantly higher methylation frequencies compared with healthy controls [64]. The motif identified in the CP patient, at − 72 bp, was identical to 1 of the 7 motifs in rheumatoid arthritis patients; however, its effect on *TNFα* expression was not examined. Recently, the incidence of coronary heart disease was found to have significant association with severe periodontitis [65]. The relationship between periodontitis and cardiovascular disease events, such as coronary heart disease, has been revealed from the view of epigenetic reciprocal interaction [66]; however, none of the experimental approaches can currently prove or clarify it. Since hyper-methylation of the CpG island was identified in *CDH1* (E-cadherin coding gene) gene loci for 25% of CP patients and in *COX2* gene loci for 19% of CP patients, which is often seen in breast cancer patients, expression levels of *CDH1* and *COX2* in periodontal tissue and the degree of methylation of the CpG islands in *CDH1* and *COX2* gene loci may be useful diagnosis markers of periodontitis [67].

Prenatal, postnatal, and immature psychological stress (i.e., early-life stress) are known to become health risk factors and cause the onset of lifestyle diseases in adulthood. Maternal deprivation, also known as maternal separation, is a representative method to evoke early-life stress in animal models [68]. Adult rats that have experienced maternal deprivation, showed severe bone loss in a ligature-induced rat periodontitis model [69]. Glucocorticoid receptor expression in the hippocampus of adult rats that have experienced maternal deprivation was upregulated concomitantly with decreased DNA methylation in the CpG site of the glucocorticoid receptor gene locus. The time-separated relation between psychological stress in early-life and periodontitis progression in adulthood suggests that early-life stress stores epigenetic memory in periodontal tissue. In the murine systemic *P.g.* challenging periodontitis model, oral gavage of *P.g.* increased the expression of DNMT3B, a de novo methyltransferase, on the surface of the alveolar bone and gut epithelial tissue. In contrast, the murine ligature-induced periodontitis model showed that expression of DNMT3B was increased only on the surface of the alveolar bone and not on the gut tissue [70]. Thus, systemic bacterial challenge is the more suitable model to analyze epigenetic influences on communication between the gut and periodontal tissues.

5. Epigenetic alteration in gingival tissue by local risk factors

Smoking increases the susceptibility and severity of CP. Combination analysis of transcriptomics and methylomics in human gingival tissue of smokers and non-smokers revealed that the genes encoding for the proteins associated with ECM organization and extracellular structure organization were selectively decreased concomitantly with increased DNA methylation in the smoker group compared with the non-smoker group [71]. Another combination analysis identified 9 hypomethylated CpG sites with significant association to current smokers compared with non-smokers and 3 of the 9 CpG sites were in the *CYP1B1* gene locus, and *CYP1B1* expression concomitantly increased in smokers compared with non-smokers [72]. *CYP1B1*, a member of the CYP superfamily, is involved in xenobiotic metabolism and endogenous metabolic pathways [73]; however, the roles of *CYP1B1* in periodontitis progression have not been evaluated. *SOCS1* was previously shown to regulate alveolar bone loss [74], and *SOCS1* promoter methylation analysis was conducted to examine whether smoking habits epigenetically altered *SOCS1* expression in oral epithelial cells [75]. *SOCS1* promoter methylation was observed in 33.3% of smoker samples and only appeared in 4.76% of non-smoker samples. Comprehensive analysis of changes in miRNA expression by nicotine treatment in PDL cells showed that the miRNA regulated Toll-like receptor signaling pathway, nicotine addiction, the transforming growth factor-β signaling pathway, and the hypoxia inducible factor-1 pathway were selectively detected compared with non-treated PDL cells [76]. These results suggest that smoking affects the local epigenetic status in periodontal tissue; however, only one pilot study (sample number = 5 per group) [71] and one epigenome-wide association study (sample number = 18 current smoker) [72] have been reported so far.

Acetylation of histone 3 was clearly detected in oral epithelial cells when periodontitis was investigated by the ligature-induced murine periodontitis model, which also detected lipopolysaccharide-treated human keratinocytes [77]. Lipopolysaccharide (LPS) suppressed the expression of DNMT1, a DNA methyltransferase, and induced the acetylation of histone 3, especially H3K9ac, and the recruitment of p300/CBP, a transcriptional co-activator, into the *NF-κB* gene locus. Furthermore, in vitro analysis of epithelial cell barrier formation showed that *P.g.*-mediated barrier destruction was blocked by several DNA methyltransferase inhibitors such as RG108, (-) epigallocatechin-3-gallate, and curcumin [78]. These results indicated that epigenetic alteration in epithelial cells by pathogens, or pathobionts exposure, is involved in the development of periodontitis, and administration of epigenetic drugs into oral epithelia should be an attractive method for preventing periodontitis.

6. Molecular mechanisms of epigenetic alteration in periodontitis

6.1. The functions of PDL cells and mesenchymal stem cells controlled by epigenetic mechanisms

Energy metabolism is strongly linked with epigenetic status [79]. The tricarboxylic acid cycle, fatty acid β-oxidation, and respiratory chain involve intermediate products of the biochemical reactions for metabolic pathways and regulate cellular differentiation through the control of histone modifications [80]. Acetyl-CoA and α-ketoglutarate levels have been shown to reflect metabolic activities [81]. Fumarate has inhibited KDM5, one of the histone Lys-specific demethylases proteins, and consequently induced active chromatin markers such as H3K27ac and H3K4me3 [82]. Comprehensive epigenetic transcriptional regulation of ECM-related genes associated with H3K27ac and H3K4me3 status has been reported in PDL cells and odontoblasts [83,84]. Exogenously supplied sodium acetate is incorporated and immediately converted to acetyl-CoA by ACSS2 and used as an acetyl group for acetylation by histone acetyltransferases (HATs) [85–87]. PPARγ is a key modulator of energy metabolism, such as lipid and glucose metabolism [88], and PPARγ positively regulates osteogenic differentiation and expression of the extracellular matrix by altering H3K27ac in PDL fibroblasts [83].

Histone acetylation markers, especially H3K9ac, H3K14ac, and H3K27ac, are positively linked with osteogenic capacities of PDL cells and the degree of histone acetylation is increased during osteogenic differentiation of PDL cells in vitro [44]. Histone is acetylated by HATs and de-acetylated by HDACs. So far, chemicals that efficiently enhance HAT activity have not been realized, but chemical inhibition of HDAC activity is a more developed strategy [44]. Trichostatin A, an HDAC inhibitor, accelerated osteogenic differentiation of PDL cells through enhancement of H3K9ac and H3K14ac [89]. p-65 is potent inhibitor of osteogenic differentiation, and BMP2-mediated ectopic bone formation [90,91]. Trichostatin A restored p-65-induced suppressive functions in MSCs and periodontal regeneration in a ligature-induced rat periodontitis model [92]. Osthole, an inducer of H3K9ac and H3K14ac, restored the inflammation-dependent regenerative capacity of PDL cells [93]. XPO5 is a key protein for nuclear export of miRNAs and pre-miRNAs processing to generate active miRNAs [94–97]. XPO5 is decreased during osteogenic differentiation of PDL cells, and the decrease of XPO5 limited the ability of miRNAs targeting *RUNX2*, which resulted in induction of osteogenesis for PDL cells [98]. miRNA-153–3p inhibited osteogenic differentiation of PDL cells by down-regulating the expression of KDM6A, a demethylase of H3K27 [99]. lncRNA *SNHG1* inhibited osteogenic differentiation of PDL cells by guiding H3K27me3 into the *KLF2* promoter to suppress the expression of *KLF2*, an inducer of the *RUNX2* transcriptional ability [100,101].

Epigenetic alteration also involves the response of PDL cells under bacterial pathogen exposure. LPS was delivered from *P.g.* hyper-methylated *RUNX2* gene locus to suppress *RUNX2* expression [102] and decrease DNMT1, a DNA methyltransferase of CpG, and increase acetyltransferase p300 and NF-κB [103]. Moreover, ascorbic acid inhibited LPS-*P.g.*-dependent p300 and NF-κB expression and increased miR-210, which is known to inhibit NF-κB signaling [104]. Considering histone modification during LPS stimulation of PDL cells [105], LPS increased the enrichment of H3K4me3, an active chromatin mark, in inflammatory gene loci and increased the enrichment of H3K27me3, a suppressive chromatin mark, in ECM and osteogenic gene loci. Jmid3, a H3K27me3 demethylase, was upregulated in PDL

cells by LPS stimulation and increased the number of Jmid3-positeive cells in rat apical periodontitis [106]. Jmid3 demethylated *IL6* and *IL12* promoter in the presence of LPS to promote the expression of *IL6* and *IL12*. Curcumin, a yellow polyphenol that contains liposome, has suppressed the LPS-*P.g.*-induced inflammation and ROS production in stem cells, while restoring the LPS-*P.g.*-suppressed p300 and DMNT1 expression, and therefore demonstrated usefulness as an epigenetic modulator for future clinical application [107].

6.2. Epigenetics associated with osteoclastogenesis

Active osteoclast formation induces alveolar bone loss in periodontitis, and epigenetic mechanisms take part in osteoclastogenesis in periodontal tissue. KDM3C, one of the histone Lys-specific demethylases, is expressed in macrophages and expression was decreased by LPS-*P.g.* treatment [108]. Loss of KDM3C has promoted NF-κB signaling, osteoclastogenesis, and alveolar bone loss in the ligature-induced murine periodontitis model [108]. KDM4B, another histone Lys-specific demethylase proteins, was increased in the epithelium of periodontal diseases and in murine calvarial sections treated with *Aggregatibacter actinomycetemcomitans* LPS [109]. In contrast to KDM3C, KDM4B positively regulated inflammation and osteoclastogenesis by suppressing KDM1B-dependent heterochromatin construction in pro-inflammatory gene loci. Histone deacetylases were classified into 4 subgroups including Class I, Class IIa, Class IIb, and Class IV [44,110]. HDAC1 and HDAC2 belong to Class I. The inhibition of deacetylase activity of HDAC1 and HDAC2 with their selective inhibitors, BRD0302 and BRD6688, respectively, reduced cytokines and chemokines secretion from TNFα-primed monocytes and suppressed RANKL-induced TNFα-primed monocyte differentiation into osteoclasts, and the effects were more pronounced if HDAC1 and HDAC2 were collectively inhibited [111]. Thus, the demethylase ability of KDM4B and deacetylase activities of HDAC1 and HDAC2 may be useful targets to suppress periodontal tissue destruction.

7. Promising approaches for epigenetic diagnosis and treatments of periodontitis

7.1. Epigenetic reprogramming of periodontal tissue residential cells into osteogenic cells

Sequential treatment of gingival fibroblasts with 5-aza-2`-deoxycytidine, an inhibitor of DNA methylation, and BMP2 in the presence of osteogenic-inducing medium has induced trans-differentiation into osteoblasts [112]. First, 5-aza-2`-deoxycytidine de-methylated hyper-methylated osteogenic gene loci such as *RUNX2* and *ALP* in fibroblasts to evoke osteogenic potential. Then, subsequent BMP2 treatment promoted osteogenic differentiation of the de-methylated cells. Co-stimulation of epithelial cell rests of Malassez with 5-aza-2`-deoxycytidine and valproic, a histone deacetylase inhibitor, was sufficient to induce the trans-differentiation into osteogenic mesenchymal-like cells [113]. These results showed that enforced epigenetic alteration is an attractive strategy to efficiently generate osteogenic cells *ex vivo* for regenerative medicine.

7.2. Promising approaches for epigenetic diagnosis of periodontitis

N^6-methyladenosine (m^6A) is a common modification in eukaryotic mRNAs and lncRNAs and plays pivotal roles for various biological processes such as RNA stability, RNA translation, and RNA export [114]. There are a total of 23 regulators for m^6A post-translational modification of RNA molecules that include 8 writer, 2 eraser, and 13 reader subtypes [114]. After analyzing the gene expression level of the 23 m^6A regulators in periodontitis samples obtained from periodontal surgery, 241 periodontitis samples were successfully divided into the 3 subtypes [114]. Periodontitis associated with mild phenotypes were accumulated in suptype-1, and periodontitis with active inflammation were accumulated in subtype-2 and − 3. *ELAVL1*, one of the m^6A erasers, was positively correlated with expression of the TNFα receptor family, and *CBLL1*, one of the m^6A writers, was negatively correlated with cytokine activity. These results indicated that m^6A modification was involved in inflammatory control in periodontitis and classification by m^6A regulators might be useful for evaluating periodontitis severity. Small extracellular vesicles (sEV) in saliva, which contain extracellular vesicles from the host and outer membrane vesicles from bacteria, are being developed for diagnosis of periodontitis [115,116]. Measurement of the 5-methylcytocine (5mC) level in salivary sEV was more precise for the assessment of periodontitis progression compared with the 5mC level in whole saliva [116]. Circular RNAs (cirRNAs) may also be promising candidate molecules for periodontitis diagnosis because the secretion of cirRNAs from periodontal tissues has been reported [117–122]. Mechanistically, cirRNAs may act as an RNA sponge, like lncRNA, that generates the cirRNAs-miRNAs-mRNAs regulatory network.

8. Conclusions

Recent advances in next-generation sequencing technologies enabled periodontal researchers to precisely explore whole-genome epigenetic changes in local periodontal tissues and peripheral blood samples of periodontitis patients. Epigenetics and epigenetic alteration have been utilized for evaluating susceptibility, diagnosis, and progressive assessment of periodontitis. However, whole-genome high-throughput screening that highlights DNA methylation and ncRNAs has only been applied for a small number of studies [35–40,71,72]. Assay for transposase-accessible chromatin sequencing (ATAC-seq) will contribute to whole-genomic high-throughput analysis in periodontitis. ATAC-seq can be used to identify whole-genomic chromatin accessibility, key histone modifications, transcriptional factors, and distal enhancer elements, which comprehensively regulate functionally related genes.

Finally, since various chemical epigenetic modulators show positive effects for protecting alveolar bone loss in vivo and osteogenic ability of PDL cells in vitro [44,46,110,123], the epigenetic status of periodontal tissue will strongly influence the responsiveness of periodontal tissue regeneration therapy. Significantly, assessment and modulation of the epigenetic status of PDL cells make it possible to evaluate and accelerate their regenerative-tropism to predict more accurate prognoses of periodontal regeneration therapy and develop precision-medicine in periodontal regeneration therapy.

Conflicts of interest

The authors declare no conflict of interest.

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