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Review Article

Emerging role of epigenetic regulations in periodontitis: a literature review

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Abstract: Periodontitis is mainly initiated by periodontal pathogens including *Porphyromonas gingivalis*, and bad living habits such as smoking aggravate its incidence and severity. The development of periodontitis is closely related to the host's immune responses and the secretion of various cytokine networks. Moreover, periodontitis has an important connection with the development of systemic diseases. Recently, epigenetics which is a fast-developing hot research area has provided new insights into the research of various diseases including periodontitis. Epigenetics is an important supplement to the regulation of gene expression. The study of epigenetics is about causing heritable gene expression or cell phenotype changes through certain mechanisms without changing the DNA sequence. It mainly includes histone modification, DNA methylation, non-coding RNA and the latest research hotspot m6A RNA methylation. In the review, we comprehensively summarize the latest literature on the potential epigenetic regulations in various aspects of periodontitis.

Keywords: Epigenetics, regulation, periodontitis, methylation

Introduction

Periodontitis is a chronic inflammatory disease caused by bacteria in dental plaque invading periodontal tissue. It can lead to periodontal destruction, periodontal pocket formation, attachment loss and alveolar bone absorption [1, 2]. Periodontitis is the main cause of adult tooth loss. According to the fourth China oral health epidemiological survey report, the detection rate of gingival bleeding among 35-44-year-olds was 87.4%, rate of dental calculus was 96.7%, and rate of attachment loss (greater than or equal to 4 mm) was 33.2% [3].

One essential process is the secretion of inflammatory cytokine networks in periodontitis. The cytokines induce the host's susceptibility and strengthen the immune response, which contribute to tissue destruction in periodontitis [4, 5]. Accordingly, numerous experimental studies have used many types of inflammation-controlling agents and factors that improve osteogenic and mineralization properties of periodontal ligament cells (PDLC) as a strategy to treat periodontitis [6-8] (**Figure 1**). It is worth noting that periodontitis is not just an oral disease, but it can potentially affect the health of other body systems, and is a risk factor for that development or aggravation of a variety of systemic diseases [9], like diabetes [10], rheumatoid arthritis [11], cardiovascular disease [12], etc.

Epigenetics refers to the interplay between epigenetic modification and the regulation of gene expression and differentiation, as well as genetic changes in gene activity or cell phenotype that occur without changing the DNA sequence [13]. Epigenetic regulation is an important way of regulating gene expression in the process of growth, development, aging and disease occurrence. The orderly response of an individual's life to environmental factors depends on to a large extent the effective operation of the epigenetic regulatory network [13].

The main epigenetic mechanisms include DNA methylation, histone modification [14], non-coding RNA molecules [15], and mRNA methylation-mediated gene expression regulation

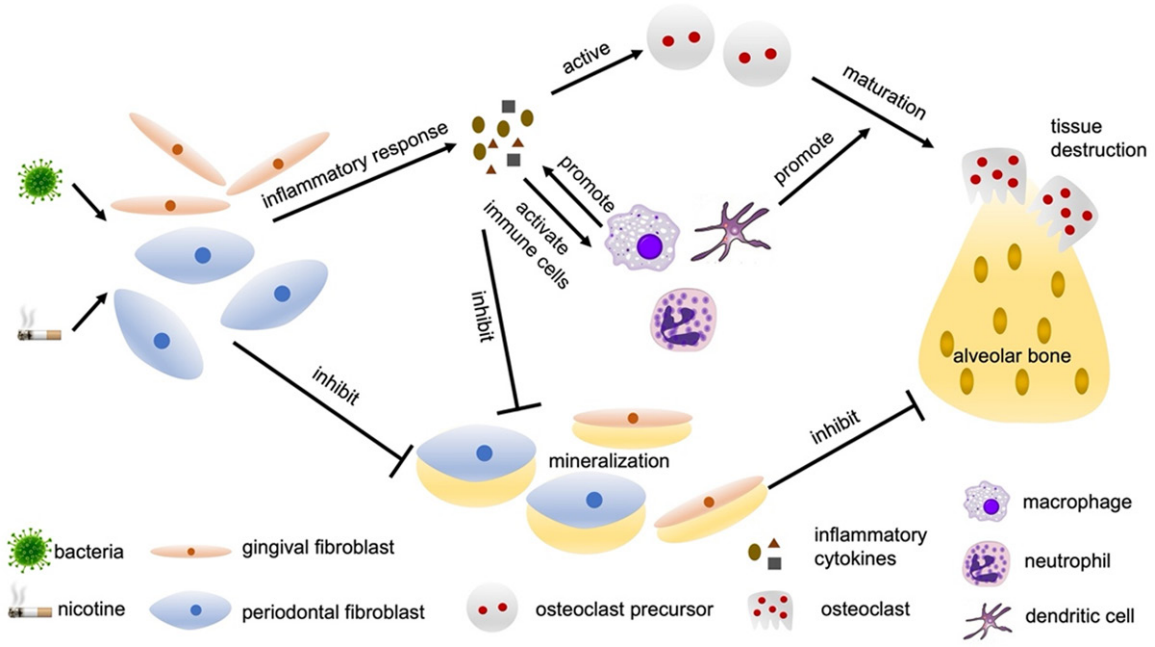

Figure 1. In periodontitis, the periodontal pathogens induce the secretion of inflammatory cytokines. The cytokines activate the immune cells and promote the differentiation of osteoclasts, which leads to tissue destruction. Also, the osteogenic abilities of the periodontal cells are inhibited under stimulations of the pathogens. The potential supplement to bone destruction is suppressed. Accordingly, the strategy to treat periodontitis can be focused on alleviating inflammatory response and enhancing the osteogenic potential of periodontal cells.

Table 1. Research methods of epigenetic regulations

Epigenetic regulation	Research methods		ref
Histone modification	liquid chromatography - tandem mass spectrometry (LC-MS-MS), Chromatin immunoprecipitation (ChIP), and ChIP-qPCR		[17, 18]
DNA methylation	Methylation analysis at overall level of the genome	DNA methylation analysis of specific sites	[19]
	high performance liquid chromatography (HPLC), methylation sensitive amplified polymorphism (MSAP), methylated DNA immunoprecipitation (MeDIP)	combined bisulfite restriction analysis (COBRA), whole genome bisulfate sequencing (WGBS)	
Non-coding RNA	RT-PCR, high-throughput sequencing, RNA-pulldown, RNA-immunoprecipitation assay		[20]

[16]. Through the response to developmental or environmental stimuli, various epigenetic regulations cooperate to enable cells to transmit genetic information and related phenotypes. The advance of research methods has provided the opportunity to precisely detect the epigenetic regulation (**Table 1**) [17-20].

This review aims to generalize the latest *in vivo and in vitro* studies regarding epigenetic regulations in periodontitis, mainly from three perspectives including histone modification, DNA methylation and non-coding RNA (**Figure 2**).

Histone modification and periodontitis

Histones assemble and arrange DNA into nucleosomes, enabling their storage in the nucleus. Each nucleosome contains two subunits, both composed of histones H2A, H2B, H3, and H4, linking histone H1 as a stabilizer. Histone H3 is frequently modified to predict the type of chromatin, distinguish the functional elements of the genome, and establish whether these elements are active or inhibited. Research on histone modifications primarily focuses on dynamic methylation [21] and acetylation [22]. Post-translational modification of histones regulates the genome into autosomal active regions available for DNA transcription. On the other hand, histone modification tightens DNA into inactive heterochromatin regions, hence, making it difficult for transcription [23].

Histone methylation

Observation of histone modification in periodontitis is relatively limited. A study performed

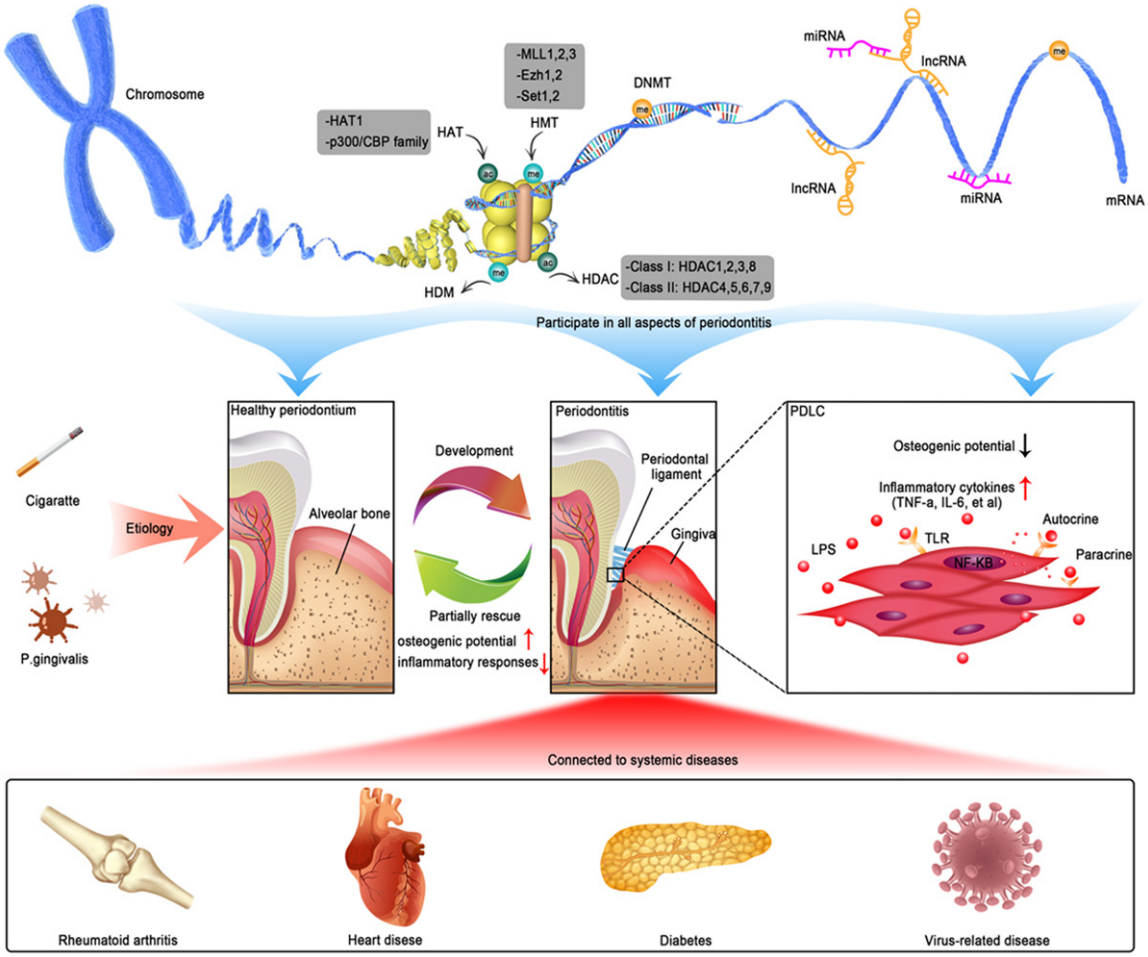

Figure 2. Epigenetic mechanisms include histone modification, DNA methylation, ncRNAs and RNA methylation. Histone modifications are mainly comprised of methylation and acetylation, which are balanced by HMT versus HDM, and HAT versus HDAC. DNA methylation is catalyzed by three DNMTs (DNMT1, DNMT3A and DNMT3B). Different ncRNAs function via competitive endogenous RNA (ceRNA) mechanism. m6A is the most frequent RNA methylation. All the mechanisms make alterations to cell phenotypes without variation of DNA sequence. All epigenetic regulations are deeply involved in the initiation, progress, therapeutic clues of periodontitis and connection to systemic diseases. Periodontal pathogenic bacterium, inflammatory cytokines and unhealthy living habits cooperate to influence the epigenetic pattern. The changes of expression of key genes induced by epigenetic modifications promote the development of periodontitis. Moreover, epigenetics may be a potential mechanism of connection between periodontitis and systemic diseases. Targeting these epigenetic molecules may have vital treatment value for periodontitis.

chromatin immunoprecipitation-on-chip analysis comparing the H3 methylation data between healthy and LPS-stimulated PDLCs. In healthy PDLCs, active H3K4me3 was highly enriched at COL1A1, COL3, and RUNX2 gene promoters, while DEFA4, CCL5 and IL-1β gene promoters were highly occupied by the repressive H3K27me3. LPS infection upregulated H3K4me3 on inflammatory responsive genes and H3K27me3 on extracellular matrix and osteogenesis lineage genes [24]. In PDLCs treated with P. gingivalis LPS, the expression of one histone methyltransferase SETD1B was elevated. Besides, increased enrichment of SETD1B and H3K4me3 on IL-1β, IL-6 and MMP2 promoter were detected via ChIP-qPCR analysis. Consequently, SETD1B knockdown decreased these inflammatory cytokines in LPS-treated PDLCs. Also, NF-κB signaling was confirmed to be involved in the process. SETD1B knockdown suppressed the p65 activation while p65 knockdown reduced SETD1B nuclear mutually. NF-κB inhibition via BOT-64 downregulated SETD1B expression and pro-

moted osteogenesis in a mouse periodontitis model [25]. LPS infection downregulated the expression of histone demethylase PHF8, in which TLR4 might be involved. The downregulation could be reversed by TLR4 inhibitor TAK-242 [26]. IGFBP5 is essential in MSC-mediated periodontitis defect regeneration since IGFBP5 promotes migration, proliferation, and osteogenic differentiation of MSCs yet suppresses inflammatory responses. Nonetheless, depletion of KDM6B or forming a protein complex of KDM6B/BCOR induced K27 methylation of IGFBP5 promoter and downregulated its expression [27, 28]. PRDM9 is an HMT, and its depletion increases the proliferation and migration of PDLCs. Mechanistically, depletion of PRDM9 elevated the H3K4me3 level in the IGFBP5 promoter and its transcription [29].

Histone acetylation

Class I and II histone deacetylase expression were detected in gingival biopsies obtained from 21 patients with chronic periodontitis and 19 control individuals. The findings showed that HDACs 1, 5, 8, and 9 were evaluated in chronic periodontitis in mRNA and protein levels [30].

Regarding treatment implications of histone modifications in periodontitis, several HDAC inhibitors have been studied. Two HDAC inhibitors were compared in mice periodontitis. Consequently, 1179.4b - an inhibitor of both Class I and II histone deacetylase was more effective than MS-275 - an inhibitor of Class HDAC only in preventing alveolar bone loss [31]. Another HDACi Trichostatin A (TSA) increased the osteogenic markers but not the adipocytic markers in PDLCs. Through immunoblotting, histone H3 at lysines K9/K14 was the acetylated target molecule of TSA [32]. What's more, TSA induced acetylated RUNX2, enhancing the osteogenic potential of PDLCs [33]. Another HDACi - sodium butyrate (NaB) was shown to enhance the osteoblast-specific markers and inhibited the secretion of inflammatory cytokines in PDLCs [34]. In another *in vitro* study using gingival fibroblasts (GFs), Pan-HDACi decreased the inflammatory mediators after stimulation. More importantly, selective inhibition of HDAC3 was essential in the suppression of the inflammatory response [35]. One histone acetyltransferase GCN5 was downregulated in PDLCs after inflammatory stimulation. Drugs upregulating GCN5 demonstrated a protective role in rat periodontitis and promoted osteogenic differentiation of PDLCs. Mechanistically, GCN5 induced acetylation of H3K9 and H3K14 at DKK1 promoter, thereby inhibiting Wnt/β-catenin signaling pathway [36].

Regarding the connection to systemic disease based on histone modification, citrullinated histone H3 was detected in the periodontal tissue of periodontitis patients. Therefore, periodontitis is considered to be a source of antigens targeted by autoantibodies against citrullinated proteins (ACPA), specific for rheumatoid arthritis (RA) [37]. In Epstein-Barr virus (EBV) - related diseases, ZEBRA is the product of the EBV BZLF1 gene and crucial for the virus transition from latency to lytic cycle. Butyric acid, culture supernatant from *P. gingivalis*, inhibited HDACs and increased histone acetylation in EBV-infected BL cells, thereby upregulating transcription of the BZLF1 gene. Then, ZEBRA expression was upregulated while EBV reactivation was induced, implying that periodontitis pathogens are a risk factor for EBV - related disease [38]. Researchers investigated the effect of metabolic by-products of short-chain fatty acids (SCFAs) on the Kaposi's Sarcoma-Associated Herpesvirus (KSHV) replication. As a result, SCFAs increased histone acetylation and decreased trimethylation to transactivate the viral chromatin [39]. Also, *P. gingivalis* was reported to be connected to reactivation of HIV-1 virus. Butyric acid was a product of *P. gingivalis* and played the role of inhibitor of HDAC. Butyric acid was demonstrated to cause histone acetylation and corepressor complex comprising HDAC1 and AP-4 was dissociated from the HIV-1 long terminal repeat promoter. This study suggested periodontal disease as a risky factor of HIV-1 reactivation from the view of histone modification [40].

Although not described in the central dogma, the histone status would affect the availability of DNA transcription. As mentioned above, the osteogenic factor RUNX2 could both be affected by histone methylation and acetylation, which might be vital for the regenerative potentials of periodontal cells.

DNA methylation and periodontitis

Among DNA chemical modifications, the most thoroughly studied is 5-methylcytosine (5mC),

which is generally regarded as a stable inhibitory regulator of gene expression [41]. The human genome contains approximately 1% methylated cytosine, hence, the most abundant and extensive DNA modification. 5mC was originally located in the CpG island, a common segment of DNA in the promoter region of genes rich in CpG dinucleotides. Within these promoter regions, 5mC acts as a stable epigenetic marker inhibiting gene transcription. In the mammalian genome, methylated cytosine is initially integrated into DNA by methyltransferases DNMT3a and DNMT3b during early development [42]. Subsequently, an additional methyltransferase, DNMT1, replicates the DNA methylation pattern to the daughter strands during DNA replication, thereby maintaining these methylation markers throughout the genome [43]. Noteworthy, 5mC was not a completely stable modification. In contrast, it is a dynamic process and DNA methylation that can be reversed by the ten-eleven translocation (TET) enzyme [44].

Plenty of studies have evaluated the DNA methylation pattern of candidate genes from clinical samples of periodontitis patients. Research has revealed a hypomethylation of STAT5 promoter [45], PTGS2 promoter [46], increased methylation at two CpG sites of TNF-α promoter [47] and higher methylation frequency of the TLR2 gene [48] in gingival tissues from chronic periodontitis patients. The methylation status of the IFN-γ, IL-10 [49] and IL-6 [50] were not significantly different in chronic periodontitis compared to the control group. In research regarding aggressive periodontitis (AgP), methylation of CCL25 and IL17C were decreased in AgP gingival tissue compared to healthy controls [51]. In oral epithelial cells from AgP patients, a higher frequency of hypomethylation of IL-8 [52], a lower level of SOCS1 demethylation, and a lower percent of LINE-1 overall methylation [53] were detected.

Moreover, advances in high throughput technology and bioinformatic tools enable an analysis of the DNA methylation in a bigger scale of candidate genes. Methylation status of 1284 immune-related and 1038 cell-cycle-related genes were analyzed on gingival samples from 12 periodontitis cases and 11 age-matched healthy individuals via Illumina. The results indicated that the mean methylation scores and the frequency of methylated probes were significantly lower in genes related to the immune process [54]. The alteration of DNA methylation must be dependent on the methyltransferase. In buccal mucosa cells from CP, the T allele and TT genotype in DNMT3B were more frequent, and miR-9-1 methylation occurred more frequently, potentially promoting CP [55]. In contrast with the periodontal lesion in gingivitis patients without alveolar bone loss, expression of TET2 enzyme was significantly enhanced in lesions of periodontitis patients. Moreover, 5-hydroxymethylcytosine (5hmC) was higher in blood than in tissues among periodontitis patients [56].

Smoking is a risk factor for the initiation and development of periodontitis. However, its role from an epigenetic view in periodontitis is unclear. The DNA methylation of TLR2 and TLR4 of gingival biopsies from 11 periodontitis smokers (PS), 11 periodontitis non-smokers (PNS), and 11 healthy controls (H) were observed. Methylation frequency of HhaI site of TLR2 gene was lower in PS and PNS group than H group, whereas TLR4 gene in all groups was mostly unmethylated [57]. SOCS1 methylation was compared between smokers with chronic periodontitis (CP/S) and non-smokers with chronic periodontitis (CP). Epithelial cells from CP/S were about seven times more likely to have a methylated SOCS1 than that from the CP group [58]. Based on research comprising 40 control individuals, 30 smokers with CP, and 40 non-smokers with CP, a lower percent of IL-8 methylation was detected in epithelial cells of CP patients with or without smoking, compared to the control group [59].

As a major periodontitis pathogen, the role of P. gingivalis LPS as an epigenetic mediator was investigated. DNMT1, HDAC1, and HDAC2 decreased after the challenge of periodontitis pathogens *P. gingivalis* or *Fusobacterium nucleatum* in GECs. Pretreatment of DNMT or HDAC inhibitor elevated the expression of antimicrobial cytokines b-defensin 2, CCL20, and IL-8 [60]. In the gingival epithelium of mice periodontitis and LPS-treated human gingival keratinocytes, DNMT1 was down-regulated, and acetylation of histone 3 (ac.H3) was induced, causing the recruitment of p300/CBP co-transcription factor, resulting in the activation of NF-κB activation [61]. Similar results were

repeated in hPDLCs [62]. However, other studies demonstrated conflicting findings. The expression of DNMT1 in hPDLCs stimulated with *P. gingivalis* LPS was significantly elevated while the methylation of RUNX2 DNA at 0.1 kb-1.9 kb was significantly higher, and expression of RUNX2 was lower, which could be reversed by DNMT inhibitor 5Aza [63]. *P. gingivalis* infection decreased transepithelial electrical resistance (TEER) in primary human gingival epithelial cells, indicating a destructed barrier function. This phenomenon might be linked to increased DNA methylation of three cell-cell junction complexes, CDH1, PKP2, and TJP1. Administration of DNMT inhibitor prevented these LPS-induced changes [64]. IL-1β and PGE2 were important inflammatory mediators in the ongoing and development of periodontitis. One study showed that exposure of human gingival fibroblasts to IL-1β upregulated the expression of maintenance methyltransferase DNMT1 but downregulated *de novo* methyltransferase expression DNMT3a and the demethylating enzyme TET1, while PGE2 downregulated expression of all three enzymes [65]. Besides the enzymes, the methylation of candidate genes was evaluated, which might be directly responsible for the effects causing periodontitis. After LPS stimulation in human periodontal fibroblasts (hPDLFs), 25 extracellular matrices (ECM)-related genes exhibited 4-fold greater hypermethylation. Among them, transcriptions of nine genes (FANK1, COL4A1-A2, 12A1 and 15A1, LAMA5 and B1, MMP25, POMT1, and EMILIN3) were significantly decreased due to the hypermethylation [66]. LPS treated HCEM revealed DNA hypermethylation in GJA1, BMP2, and BMP4, inducing decreased mineralization levels. Notably, 5Aza abolished the LPS-induced decrease [67]. *Treponema denticola* is another risk pathogen for periodontitis, and its challenge in PDLCs caused a detectable decrease in methylation of the MMP-2 promoter and induced MMP-2 expression, causing matrix degradation and bone resorption [68].

Conventional clinical periodontal therapy includes the removal of supragingival calculus and subgingival instrumentation. Studies have evaluated the impact of periodontal therapy on the DNA methylation status of biopsies, confirming the effect of DNA methylation on periodontitis development from another perspective. Gingival biopsies were collected from healthy individuals and CP patients three months after periodontal therapy. The methylation profiles of SOCS1, SOCS3, and LINE-1 genes were similar between two groups in epithelial and connective tissues [69]. In another study, DNA profiles of gingival biopsies were observed in healthy and periodontitis patients at baseline and at 2 and 8 weeks after treatment. The results demonstrated that periodontal therapy re-set the DNA methylation status of COX-2 in periodontitis patients. In detail, the methylation percentage of COX-2 decreased significantly at 2 and 8 weeks after therapy and was close to the level in the healthy group. However, periodontal therapy demonstrated little effects on the methylation status of TNF-α, IFN-γ and LINE-1 [70].

As to treatment implications from the view of epigenetic regulation, some *in vitro* studies offered a new insight. TGF-β is an anti-inflammatory mediator, inhibiting NF-κB signaling pathway and decreasing the secretion of TNF-α and IL-8. The anti-inflammation effect of TGF-β depended on protein arginine methyltransferase I (PRMTI) - mediated Smad6 methylation. Mechanistically, methylated Smad6 could mediate MyD88 degradation, thereby suppressing NF-κB activation [71]. Knockdown of DNA demethylases-Tet 1 and 2 led to Dkk1 hypermethylation and activated WNT signaling pathway, thus increasing expression of Fas ligand. The molecular changes above enhanced the immunomodulation of periodontal ligament stem cells (PDLSCs) and enhanced the PDLSCs-mediated amelioration of colitis in mice [72]. This might be an implication of periodontitis amelioration.

Concerning the role of DNA methylation in the connection of periodontitis and systemic disease, rheumatoid arthritis and diabetes have been studied. The 19 CpG motifs of the IL-6 gene promoter were analyzed in peripheral blood from 30 RA patients, 30 CP patients and 30 healthy individuals. The results showed IL-6 methylation levels of the CpG motif at -74 bp were lower in the RA and CP group than controls respectively, inducing higher IL-6 concentration [73]. Another study compared 12 CpG motifs of TNF-α in peripheral blood from 30 RA patients, 30 CP patients and 30 healthy individuals in Japan. Unlike the healthy group, a

higher methylation rate and the frequency at -72 bp was observed in the CP group. Seven CpG sites (-302, -163, -199, -72, -49, -38 and +10 bp) were more highly methylated in the RA group than the healthy group [74]. In investigating the correlation between periodontitis and cancer in DNA methylation pattern, one study analyzed the methylation of E-Cadherin and COX-2 in blood samples from 108 healthy subjects, 110 periodontitis and 106 breast cancer patients. However, none was detected among healthy individuals; hypermethylation of E-Cadherin and COX-2 was reported at 38% and 35% in breast cancer and 25% and 19% in periodontitis [75].

Epithelial cells of buccal mucosa were obtained from 21 control subjects (C), 29 periodontitis patients (P) and 22 diabetes/periodontitis patients (D/P) and methylation form of CXCL12 was analyzed. The findings showed no significant differences in CXCL12 methylation among the three groups. Whereas DNA methylation of CXCL12 was significantly correlated to periodontal parameters and glycosylated hemoglobin separately [76]. The genome-wide DNA methylation pattern was analyzed in a mini pig model with streptozotocin-induced diabetes. STZ-induced diabetic status and altered the methylated expression of 1163 genes, where 599 and 564 genes were significantly hyper- and hypo-methylated. GO term analysis showed that genes responsible for biological process occupied the largest part. KEGG pathway analysis demonstrated that the genes were related to various aspects of inflammation-related signaling [77]. High glucose enhanced the expression of DNMT and consequently higher DNA methylation in diabetic rats and in human PDLCs. Additionally, HG impaired the osteogenic differentiation capacity of hPDLCs and could be rescued by DNMT inhibitor 5-aza-dC [78]. Controversially, another study demonstrated that DNMT1 expression was suppressed under high glucose condition in PDLCs. The CpG island within the TNFR-1 gene was hypomethylated and the expression of TNFR-1 was elevated, hence reducing cell viability [79].

As the initiation of genetic central dogma, DNA could be methylated by methylases without affecting its sequence. As a sure consequence, the downstream mRNA transcription and protein translation would be affected. In periodontitis, alteration of DNA methylation status of plenty of inflammation-related genes was investigated, which might be the mechanism of elevated inflammatory cytokines.

Non-coding RNA and periodontitis

Non-coding RNAs (ncRNAs), mainly including microRNAs (miRNAs) and long non-coding RNA (lncRNAs), will not be translated into protein, but play critical roles in epigenetic mechanisms and regulating genome imprinting, nuclear and cytoplasmic transport, gene transcription and clipping [80].

microRNA

miRNA is a highly conserved non-coding segment of RNA comprising 17-25 nucleotides and binds to the 3' UTR of mRNA transcribed by a target gene. In combination, the target mRNA would be degraded, or translation would be inhibited, causing a negative regulation of the target gene expression [81].

Research on the role of microRNAs in periodontitis has significantly matured, and attributed to the advances and accessibility of related technologies. Microarrays were used to detect the expression of a series of microRNA [82]. Microarrays were conducted using RNA derived from periodontitis or healthy gingival tissue in people from different countries [83-86]. Numerous microRNAs were found to be differentially expressed in these studies, most of which were functionally related to inflammatory responses, cell homeostasis, etc. Additionally, serum microRNA might serve as a biomarker of diseases [87, 88]. Serum microRNA profiles were analyzed in healthy and periodontitis groups via microarray analysis. More than two thousand microRNAs were identified differentially expressed between two groups. The up-regulation of miR-664a-3p, miR-501-5p, and miR-21-3p were confirmed by real-time PCR and these three microRNAs were considered as candidate serum biomarkers for periodontitis patients [89]. In LPS-stimulated PDLCs, 22 up-regulated and 28 down-regulated microRNAs were detected via microRNA array. GO and KEGG analysis demonstrated that these miRNAs were associated with inflammation-related pathways [90]. Defective osteogenic potentials of PDLCs were closely related to periodontitis [36]. Two studies observed the

microRNA profile in the process of osteogenic induction in PDLCs [91, 92]. Among them, downregulation of miR-24-3p promoted osteogenic differentiation of human periodontal ligament stem cells by targeting SMAD family member 5 [92]. Through re-analysis of three previous microRNA microarray files [84-86], miR-144-5p was upregulated in the three studies and verified in gingival tissue biopsy from periodontitis group [93]. Cyclooxygenase 2 (COX2) and interleukin-17F (IL17F) were predicted to be target genes of miR-144-5p and were down-regulated in periodontitis gingival samples [93].

Numerous specific microRNAs and their target genes have been studied; among them, miR-146a is a star molecular. miR-146a is widely researched due to its negative regulation of inflammatory responses by inhibiting the NF-κB signaling pathway. Its dysregulation was implicated in various immune-related and inflammatory diseases, including rheumatoid arthritis [94], Sjögren's syndrome [95], systemic lupus erythematosus [96], etc. Similarly, the expression of miR-146a was dysregulated in both gingival tissue [97, 98] and plasma [99] of periodontitis patients compared to healthy control and was closely related to the disease severity. The *in vitro* cell assays demonstrated similar results. The expression of miR-146a was elevated in LPS or *P. gingivalis* stimulated periodontal ligament fibroblasts [100, 101]. Overexpression of miR-146a or miR-146a mimics negatively regulated the inflammatory responses [101, 102]. As to the mechanism, miR-146a was bound to the 3'-UTR of TRAF6 [101]. In addition, miR-146a promoted osteogenic differentiation of PDLC via decreasing NF-κB signaling [103] and rescued the LPS-inhibited osteogenic differentiation [104]. The above findings strongly imply a protective role of miR-146a to periodontitis by down-regulating inflammatory responses and promoting osteogenic differentiation.

Additional studies investigated some specific microRNAs in various aspects of periodontitis. microRNA-205-5p was downregulated in periodontal tissue from clinical patients and in *P. gingivalis*-stimulated gingival epithelial cells. IL6ST (interleukin 6 signal transducer) was predicted via TargetScan and verified via dual luciferase reporter assay to be the target gene of miR-205-5p. Additionally, pJAK2 and p-STAT3 were significantly upregulated in *P. gingivalis*-stimulated GECs [105]. miR-23a was upregulated in gingival crevicular fluid samples from periodontitis patients compared to control subjects. The role of miR-23a involved inhibiting osteogenic differentiation of PDLCs by targeting bone morphogenetic protein receptor type 1B (BMPR1B) [106]. The expression of let-7a and miR-21 was upregulated, while miR-100 and miR-125b were downregulated in gingival tissue samples from periodontitis patients. NFKB was predicted as a common target of the four microRNAs [107]. The analysis of serum from periodontitis patients demonstrated that miR-142 expression was positively correlated with TNF-α. In GEC, TNF-α stimulation induced an upregulated expression of miR-142, which targeted basic leucine zipper transcription factor 2 (BACH2) downstream, causing apoptosis of GEC [108]. miR-21 was increased both in periodontal ligament tissue of periodontitis patients and gingival tissue of mice periodontitis models, which was a potential feedback mechanism of infection protection. miR-21 mimic inhibited the inflammatory responses in LPS-treated macrophages, while the absence of miR-21 increased NF-κB signaling [109].

Regarding the role of smoking in periodontitis from the perspective of microRNA, nicotine upregulated the expression of miR-30a and suppressed the proliferation of PDLCs. The mechanism might be that miR-30a targeted the 3' UTR of cyclin E2 (CCNE2), which was verified by luciferase reporter assay [110]. In another study, higher miR-18b was detected in PDLCs from smokers and nicotine-stimulated PDLCs. miR-18 inhibitor was able to partially reverse the suppressive effect on proliferation and migration of nicotine [111].

An *in vitro* inflammatory cell model by *P. gingivalis* was commonly used in microRNA-related research. miR-203 was elevated in GECs after *P. gingivalis* infection. SOCS3 and SOCS6 were targets of miR-203, which were decreased after infection. In downstream, Stat3 was dysregulated and host signaling responses were modulated [112]. In *P. gingivalis* LPS-stimulated PDLCs, miR-212-5p was down-regulated. Overexpression of miR-212-5p was able to inhibit the inflammatory response. The mecha-

nism might be targeting Myd88 and inactivation of MAPK and NF-κB signaling [113].

In addition, the osteogenic potential of periodontal cells was closely correlated to development of periodontitis and was a vital therapeutic target of periodontitis [6-8]. Several studies investigated the changes of a few microRNAs after the osteogenic induction of PDLCs. The results demonstrated the decrease of miR-214 [114], 21 [115], 132 [116], 125b [117] and 152-3p [118] in the process of osteogenic differentiation of PDLCs. These four microRNAs negatively regulated the osteogenic potentials of PDLCs by targeting their downstream genes. In contrast, miR-543 was upregulated during osteogenic induction of PDLCs and overexpression of miR-543 positively promoted the process [119]. Osteogenic induction under inflammatory conditions was a more natural microenvironment like periodontitis. Inflammatory cytokines inhibited osteogenic potentials of PDLCs, which might be involved with upregulation of miR-148a [120] and 138 [121] and down-regulation of miR-21 [122]. In another periodontal cell-OCCM, upregulation of miR-155-3p was responsible for inflammation-inhibited cementoblast differentiation [123]. Moreover, it was revealed that microRNAs interplay with HDAC in the osteogenic differentiation of PDLCs. miR-22 increased the expression of osteogenic markers via targeting HDAC6 [124]. Under inflammatory stimulation, the expression of HDAC9 was significantly increased. HDAC9 could be enriched on the promoter of miR-17, thereby preventing the expression of miR-17. While inhibiting the expression of HDAC9 prevented the bone loss in periodontitis. The effect was partially dependent on miR-17 [125].

There were several studies investigating the potential connection between periodontitis and systemic diseases based on microRNAs. In a study of serum and gingival crevicular fluid from periodontitis patients with or without type 2 diabetes, the level of miR-223 was increased while miR-200b was decreased in the diabetes group. These data implied that microRNA might be a biomarker of periodontitis with type 2 diabetes [126]. In another study, miR-214 was upregulated in gingival tissue of diabetes-associated periodontitis patients. The *in vitro* cell assay demonstrated that miR-214 and its target gene (activating transcription factor 4) ATF4 regulated the process of necroptosis [127]. High glucose increased the apoptosis of PDLCs by reducing the level of miR-221 and 222 and elevating caspase-3 [128]. Different microRNA profiles (like miR-15a, 18a, 22, et al) were observed between periodontitis individuals and obese periodontitis individuals. Predicted target genes of the above microRNAs were related to the expression of cytokines, chemokines and regulators of glucose and lipid metabolism [129]. In hyperlipidemic and proatherogenic ApoE-/- mice, polymicrobial periodontal pathogens including *P. gingivalis*, *T. forsythia* and *T. denticola* infection were conducted. miR-146a was found to be increased in the maxillary periodontium and spleen in mice, which functioned as a negative regulator of the inflammatory response [130]. In rats infected with the above three pathogens, dysregulation of miR-155, 132 and 146a was detected in salivary glands and pancreas. The results suggested a novel insight into the connection between periodontal pathogens and Sjögren's syndrome [131].

Long non-coding RNA

Long non-coding RNA is a kind of ncRNA with a length of more than 200 nucleotides. Its mechanism of action is complicated and has not yet been fully understood. According to the current research, lncRNAs have the following functioning mechanisms: Firstly, it forms a complementary double-strand with the transcript of the gene encoding protein, which interferes with the splicing of mRNA. Secondly, it inhibits RNA polymerase or mediates chromatin remodeling and histone protein modification. Thirdly, it works as a precursor molecule of miRNA [132].

High-throughput sequencing including microarray [133-135] and bioinformatic analysis [136, 137] were used to detect the expression profile in periodontitis. Hundreds and thousands of lncRNAs were modified and lncRNA-microRNA-mRNA networks were predicted. In periodontal tissue specimens from periodontitis patients, levels of lncRNA AC0001207, MZF1-AS1, FGD5-AS1 and OIP5-AS1 were downregulated, while levels of lncRNA RP1129G83 [138] and LINC-01126 [134, 139] were upregulated. LINC0-1126 inhibited proliferation and facilitated inflammatory responses of PDLCs by sponging miR-518a-5p [134]. Meanwhile, LINC01126

suppressed the migration of PDLCs via MEK/ERK signaling [139]. The decreased expression of FGD5-AS1 was in line with other findings [140]. Overexpression of FGD5-AS1 alleviated the LPS-induced inflammatory responses via downstream miR-142-3p/SOCS6 [140]. lncRNA taurine-upregulated gene 1 (TUG1) was decreased in periodontal tissues derived from periodontitis patients, and its overexpression played a protective role in LPS-induced proliferative inhibition and apoptosis promotion via sponging miR-132 [141]. Similarly, TUG1 was downregulated in LPS-stimulated PDLCs *in vitro*. TUG1 inhibited the inflammatory responses via regulate miR-498 and its target gene RORA [142]. lncRNA brain-derived neurotrophic factor-antisense (BDNF-AS) was decreased while PNKY was increased in periodontal tissues from periodontitis individuals [143]. In PDLCs derived from periodontitis-affected teeth, the downregulation of lncRNA MAFG-AS1 [144], papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) [145], Linc-RNA Activator of Myogenesis (Linc-RAM) [146] and mortal obligate RNA transcript (MORT) [147] was observed, whereas lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) [148] was upregulated. The common effect of the five above lncRNAs was regulating the proliferation of PDLCs [144-148]. Another downregulated lncRNA POIR made an impact on osteogenesis in PDLCs from periodontitis patients. POIR competed with miR-182 and led to depression of FoxO1 [149]. Besides periodontal tissues, lncRNA profile of peripheral blood was explored. The level of lncRNA AWPPH was elevated in plasma of periodontitis patients, which reduced after periodontitis treatment [150]. Upregulation of lncRNA p50-associated COX-2 extragenic RNA (PACER) [151] and downregulation of antisense noncoding RNA in the INK4 locus (ANRIL) [152] were detected in peripheral blood from periodontitis patients.

In a cell model simulating periodontitis, expression of lncRNA MALAT1 was upregulated in human gingival fibroblasts after *P. gingivalis* LPS or *E. coli* LPS treatment. MALAT1 promoted development of inflammation by binding with miR-20, which increased the expression of TLR4 [153]. In nicotine-stimulated PDLCs, lncRNA NEAT1 and IL-8 was significantly upregulated [154].

Like microRNA, lncRNA is widely studied in the osteogenic differentiation of periodontal cells, closely related to the development and therapeutic implies of periodontitis. The levels of lncRNAs including MEG8, MIR22HG [155], growth arrest specific transcript 5 (GAS5) [156], TWIST1 [157], X-inactive specific transcript (XIST) [158], TUG1 [159, 160], prostate cancer-associated ncRNA transcript-1 (PCAT1) [161] and Fer-1-like family member 4 (FER1L4) [162] were upregulated after osteogenic induction of PDLCs. These above lncRNAs promoted osteogenic differentiation of PDLCs. Mechanically, GAS5 enhanced osteogenic potentials by upregulating the expression of GDF5 and activating JNK and p38 signaling [156]. lncRNA TWIST1 promoted osteogenic differentiation of PDLCs by enhancing the level of TWIST1 mRNA [157]. XIST played its positive role in osteogenesis by sponging microRNA-214-3p [158]. TUG1 made the effect via sponging miR-222-3p [160]. lncPCAT1 interacted with miR-106-5p, targeting BMP2 [161]. FER1L4 sponged miR-874-3p, which target a crucial osteogenic gene VEGFA [162]. In contrast, lncRNA anti-differentiation noncoding RNA (ANCR) [163], DANCR [164] and MEG3 [165, 166] were decreased during osteogenic induction. ANCR suppressed bone formation via sponging miR-758 [163], while downregulation of ANCR increased osteogenic potential [163, 167]. MEG3 influenced osteogenesis of PDLCs via regulating BMP2 [166] or miR-27a-3p/IGF1 axis [165].

Regarding the role of lncRNA in connection with periodontitis and systemic diseases, ANRIL and cardiovascular diseases were the hotspots. ANRIL was identified as a shared genetic susceptibility locus for periodontitis and coronary heart disease (CHD) [168]/cardiovascular disease (CVD) [169]/atherosclerotic cardiovascular disease (ACVD) [170]. Decreased ANRIL led to repression of ADIPORI, VAMP3 and C11ORF10. A region upstream of VAMP3 within CAMAT1 was demonstrated to be associated with high risk of coronary artery disease and periodontitis [171]. ANRIL polymorphism was related to the elevated level of C-reactive protein (CRP), which was a risk marker for ACVD [170]. In addition, gene polymorphism influenced the expression of another lncRNA CDKN2BAS, which was involved in the

disease predisposition of periodontitis and coronary artery disease [172].

CircularRNA

CircularRNA (circRNA) is a special type of ncRNA molecule. Unlike traditional linear RNA terminated with 5' caps and 3' tails, circRNA molecule has a closed loop structure and is not affected by RNA exonuclease. Its expression is more stable and not easily degraded [173]. In terms of function, recent studies have shown that circRNA molecules act as miRNA sponges, thereby releasing miRNA's inhibitory effect on its target genes and increasing expression target genes, which is called the competitive endogenous RNA (ceRNA) mechanism [174].

In most recent years, research has investigated the role of some circRNAs in periodontitis and their related ceRNA mechanism. Compared to healthy controls, levels of circ_0085289 [175], CDR1as [176] and 0081572 [177] were downregulated and circMAP3K11 [178] was upregulated in periodontal tissues of periodontitis patients. *In vitro* dual luciferase reporter assay, RNA pull-down and RIP assay demonstrated that the above four circRNAs involve in the pathology of periodontitis following the ceRNA mechanism. To be specific, circ_0085289 alleviated LPS-induced inflammatory cytokine secretion and apoptosis, by sponging microRNA let-7f-5p and increasing downstream target gene SOCS6 [175]. Similarly, circ_0081572 prevented cell injury via miR-378h/RORA axis [177]. CircMAP3K11 promoted cell proliferation and migration, and inhibited cell apoptosis of PDLCs by targeting miR511-3p/TLR4 axis [178]. Overexpression of circCDR1as rescued the LPS-induced proliferative inhibition in PDLCs via targeting miR-7, whereas knockdown of CDR1as further promoted the inhibitory effect [176].

During osteogenic differentiation of PDLCs from D0 to D14, the expression profiles of circRNAs, miRNAs and mRNAs were significantly varied by sequencing analysis [179]. The upregulation of circMAN1A2, CRKL and RIM51 from D0 to D7 and downregulation of circETFA from D0 to D14 were verified by qRT-PCR [179]. In periodontitis tissues, the level of circCDK8 was upregulated. CircCDK8 is involved in the pathology of periodontitis via inducing autophagy and inhibiting osteogenic differentiation of PDLCs [180]. CircCDR1as was increased during osteogenic induction of PDLCs and promoted osteogenic potentials via sponging miR-7 and upregulating its target gene growth differentiation factor 5 (GDF5) [181].

Due to the convenience of detection methods, the number of altered ncRNAs was tremendous. The function of plenty ncRNAs were even udiscovered and more in deepth studies weare needed to reveal them. Additionally, it was hard to tell wether the alterations of many ncRNA expressions were the reasons or outcomes for periodontitis development. Still, due to the simpleness of observation, ncRNA alterations had higher potentials to be the biomarkers of periodontitis compared to enzymes and molecules involved in histone modification and DNA methylation. But more studies are definitely needed.

RNA methylation and periodontitis

The most common internal modification of mRNA includes N6-adenylate methylation (m6A) [182]. In the transcription process from DNA to RNA, adenylate undergoes methylation modification at the sixth position under the action of the methylases METL3, METL14 and WTAP. The bases that have been modified by m6A are able to be demethylated by the two enzymes FTO and ALKBH. These RNA base sites undergoing methylation require specific enzymes for recognition to be functional. The YTHDF family primarily includes YTHDF1, YTHDF2, and YTHDF3. These enzymes recognize the bases undergoing m6A methylation, participate in the downstream translation, mRNA degradation, and accelerate the mRNA exit rate [183].

Notably, studies on the roles of m6A in periodontitis are limited. As such, through analysis of genome-wide association studies on periodontitis, m6A-associated single-nucleotide polymorphisms might be potential functional variants for periodontitis [184]. Another microarray data study was obtained from 69 healthy periodontal samples and 241 periodontitis samples [185]. Seventeen of twenty-three m6A regulators were detected in altered expression of periodontitis, among which 15 m6A regulators were essential for periodontitis via a serious bioinformatic algorithm. Then correlation

Table 2. Epigenetic changes in various clinical samples from periodontitis patients

Epigenetic regulation		Sample	Results
DNA methylation		gingival tissues	hypomethylation of STAT5 promoter [45], PTGS2 promoter [46], increased methylation at two CpG sites of TNF-α promoter [47] and higher methylation frequency of TLR2 gene [48] in CP
		gingival tissue	methylation of CCL25 and IL17C were decreased [51] in AgP
		oral epithelial cells	a higher frequency of hypomethylation of IL-8 [52], a lower level of SOCS1 demethylation, and a lower percent of LINE-1 overall methylation [53] in AgP
		gingival biopsies	methylation frequency of HhaI site of TLR2 gene was lower in periodontitis [57]
		epithelial cells	a lower percent of IL-8 methylation in CP than healthy group [59], a higher methylated SOCS1 in smokers with CP than non-smokers with CP [58]
Non-coding RNA	microRNA	serum	up-regulation of miR-664a-3p, miR-501-5p, miR-21-3p [89] and miR-142 [108]
		gingival tissue	up-regulation of miR-144-5p [93], miR-146a [97, 98], let-7a and miR-21 [107], down-regulation of miR-100, miR-125b [107]
		plasma	down-regulation of miR-146a [99]
		periodontal tissue	down-regulation of miR-205-5p [105]
		gingival crevicular fluid	up-regulation of miR-23a [106]
	lncRNA	periodontal tissue	down-regulations of lncRNA AC0001207, MZF1-AS1, FGD5-AS1, OIP5-AS1 [138] and BDNF-AS [143], up-regulations of lncRNA RP1129G83 [138], LINC01126 [134, 139], TUG1 [141] and PNKY [143]
		PDLCs	downregulation of lncRNA MAFG-AS1 [144], PTCSC3 [145], Linc-RAM [146] and MORT [147], up-regulation of lncRNA MALAT1 [148]
		peripheral blood	up-regulation of lncRNA AWPPH 150, PACER 151, downregulation of ANRIL 152
	cirRNA	periodontal tissue	down-regulation of circ_0085289 [175], CDR1as [176] and 0081572 [177] and up-regulation of circMAP3K11 [178]
RNA methylation		periodontal tissue	expressions of 17 m6A regulators were altered, and were closely related to immune microenvironment [185]

Table 3. Epigenetic changes in cell assay related to periodontitis

Epigenetic regulation		Cell type	Stimulation	Result
Histone methylation		PDLC	LPS	Upregulation of H3K4me3 on inflammatory responsive genes and H3K27me3 on extracellular matrix and osteogenesis lineage genes [24], down-regulation of the expression of histone demethylase PHF8 [26]
		PDLC	*P.g* LPS	increased enrichment of SETD1B and consequent upregulation of H3K4me3 on IL-1β, IL-6 and MMP2 promoter [25]
DNA methylation		GEC	*P.g Fusobacterium nucleatum*	or Expressions of DNMT1, HDAC1, and HDAC2 decreased [60]
		gingival keratinocytes or PDLC	LPS	DNMT1 was down-regulated, and acetylation of histone 3 (ac.H3) was induced [61, 62]
		PDLC	*P.g* LPS	expression of DNMT1 elevated and methylation of RUNX2 DNA was higher [63]
		GEC	*P.g*	Increased DNA methylation of CDH1, PKP2, and TJP1 [64]
		Gingival fibroblast	IL-1β	Upregulation of DNMT1 but downregulation of DNMT3a and TET1 [65]
		PDLC	LPS	25 extracellular matrices (ECM)-related genes exhibited 4-fold greater hypermethylation [66]
		HCEM	LPS	DNA hypermethylation in GJA1, BMP2, and BMP4 [67]
non-coding RNA	microRNA	PDLC	LPS	up-regulation of miR-146a [100]
		PDLC	*P. g*	up-regulation of miR-146a [101] and down-regulation of miR-212-5p [113]
		GEC	*P. g*	down-regulation of miR-205-5p [105] and up-regulation of miR-203 [112]
		PDLC	nicotine	up-regulation of miR-30 [110]
	lncRNA	PDLC	LPS	down-regulation of lncRNA TUG1 [142]
		gingival fibroblast	*P.g* LPS	up-regulation of lncRNA MALAT1 [153]
		PDLC	nicotine	up-regulation of lncRNA NEAT1 [154]

analysis found a close relation between dysregulated m6A and immune microenvironment, including infiltrating immunocytes, immune reaction gene-sets and HLA gene expressions. Three distinct subtypes of periodontitis could be distinguished via unsupervised consensus clustering analysis according to the expression patterns of m6A regulators.

Conclusion and perspective

In addition to the genetic information carried by the DNA sequence itself, the epigenetic regulations have become a vital link between the external environmental factors and the regulation of gene transcription [186].

In terms of periodontitis, periodontal pathogenic bacterium, inflammatory cytokines, and unhealthy living habits cooperated to promote the progress of the disease. In various clinical samples, including periodontal tissue and peripheral blood, alteration of epigenetics-related profiles was detected (**Table 2**). Expression patterns of enzymes responsible for histone modification, DNA methylation and ncRNAs were different in periodontitis and healthy individuals. These data implied that these molecules may be potential biomarkers of periodontitis. However, translational application of the research still has a long way to go. A larger number of clinical samples are needed to confirm accurate biomarkers. *In vitro* cell assays and *in vivo* animal periodontitis models revealed the molecule mechanism and signaling pathway of epigenetic changes induced by *P. gingivalis*, LPS, nicotine, etc (**Table 3**).

On the other hand, the changes in expressions of key genes induced by epigenetic modifications promote the development of periodontitis. Targeting these epigenetic molecules may result in inhibiting the inflammatory response and promoting osteogenic potentials of PDLCs, which have important clinical value for the treatment of periodontitis.

The link between periodontitis and systemic diseases were discussed from various aspects [187]. Epigenetic mechanisms are new connection bridges and they influence immune responses and key molecules [188].

However, despite the abundant research into the histone modification, DNA methylation and ncRNAs in periodontitis, publications of m6A RNA methylation in periodontitis are deficient. Current research has demonstrated that m6A was involved in tooth root formation [189], cell cycle of dental pulp stem cells [190] and inflammation of dental pulp cells [191]. It's reasonable to speculate that m6A may be involved in periodontitis.

The literature included in the present review adequately elaborated the epigenetic regulations of gene expressions without alteration of DNA sequences. It described a network containing stimulation, epigenetics mechanisms, modifications of gene expressions and outcomes in periodontitis. The network offered additional information into the mechanisms of periodontitis development and accordingly it provided new insights of periodontitis therapy ideas.

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Disclosure of conflict of interest

None.

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