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Epigenetics And its Role in The Pathogenesis of Periodontal Disease

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Abstract: *Epigenetics is the study of mitotically and meiotically heritable changes in gene function that are not dependent on DNA sequence. Epigenetic changes are reversible unlike genetic changes and are influenced by environmental factors. Epigenetic modifications include DNA methylation, modifications of histone protein structure and posttranslational repression by micro-RNA which contribute to alterations in gene expression. The rapidly evolving field of epigenetics help us to understand better the gene-environment interactions. This article aims to review the possible role of epigenetic modifications in periodontal disease pathogenesis.*
Keywords: *DNA methylation, Epigenetics, Gene regulation, Histone modification, Periodontitis*

I. Introduction

Epigenetics is the study of the epigenome. Epigenome, which is the overall epigenetic state of an organism, is as important as the genome for normal development. Environmental factors can have profound effects on the epigenetic changes and induce susceptibility to disease[1].There are three types of epigenetic modifications seen in mammalian cells – DNA methylation, histone modification and RNA- associated silencing (micro- RNA)[2]. Of these, the enzymatic DNA methylation of the C-5 position of cytosine residues in the CpG islands of the promoter region of a gene is considered to be the most important epigenetic mechanism in mammals[3].These epigenetic modifications lead to remodelling of the chromatin and activation or inactivation of a gene.The emerging evidence show that epigenetic modifications play a crucial role in the pathogenesis of various infectious and inflammatory diseases including cancer[4].Some epigenetic modifications are reversible and can be induced and/or altered by environmental factors such as diet, smoking and age there by presenting a link between the inherited genome and the environment[5].The growing knowledge about epigenetics may provide explanations as to why patients with the same clinical phenotype respond differently to treatment.

Periodontitis is a multifactorial disease characterized by chronic inflammation in the gingival tissues in response to bacteria colonizing the tooth surface, ultimately leading to tissue destruction with loss of alveolar bone and connective tissue[6].The epithelial cells in the oral cavity are constantly being exposed to high levels of various bacteria and the resulting host inflammatory response is influenced by both genetic and environmental factors[7].A specific gene can possess different epigenetic patterns depending on the cell type which results in local and systemic expression of the gene. This specifies a local, site-specific change in the immune response to external stimuli and/or inflammatory response, which may differ between individuals and add to the susceptibility of the disease. This review focuses on the epigenetic modifications of the DNA and the chromatin and how they contribute to the pathogenesis of periodontal disease.

II. Dna Methylation And Periodontitis

In periodontitis, during inflammation, epigenetic modifications occur locally at the biofilm- gingival interface around the teeth. It has been reported that different methylation patterns associated with pathways regulating cell differentiation, apoptosis, lipopolysaccharide mediated signalling, oncogenesis and cell adhesion are found in inflamed tissue from periodontitis patients when compared to tissue from healthy individuals[8].When the DNA methylation pattern of TLR2 and TLR4 genes were evaluated in gingival tissue samples, TLR4 gene promoter was unmethylated in most samples, while TLR2 promoter was both methylated and unmethylated[9].A positive correlation was found between TLR2 methylation and periodontal probing depth. A study on Aggressive periodontitis showed an overall demethylation pattern of the SOCSI gene. In healthy subjects, this methylated level was higher. A significant difference in the methylation pattern for the LINE-1 gene was also reported[10].When the methylation status of E-Cadherin and COX-2 genes was investigated in chronic periodontitis and breast cancer patients, a hypermethylation of these two genes was found in both these patients compared to healthy individuals[11].The findings of this study confirm the present view that chronic inflammation and cancer may have a similar epigenetic pattern and/or that DNA methylation may be a link between inflammation and cancer[12].Studies have shown that enzyme that cause methylation can be regulated

directly or indirectly by the extracellular milieu through signal transduction pathways such as the MAPK ERK pathway[13].Inflammatory processes may result in aberrant DNA methylation in tissues. This mechanism has been suggested as a link between inflammation and methylation levels in cancer. A study evaluated the levels of TET1, TET2, and 5hmC in CD4+T cells in Systemic lupus erythematosis (SLE) patients compared to healthy individuals[14].A high level of these markers were seen in SLE patients suggesting that DNA hydroxymethylation may be contributing to the pathogenesis of SLE. Since SLE and periodontitis share some common features regarding immune response, it may be that DNA hydroxymethylation plays a role in periodontitis similar to that of SLE.

III. Epigenetic Changes In Cytokine Genes In Periodontitis

Several studies have been conducted in the recent years on epigenetic changes in the cytokine genes implicated in periodontitis pathogenesis. A study on epigenetic changes in subjects with chronic periodontitis, the methylation status of DNA in the promoter region of interleukin-8 (IL-8) in gingival and oral mucosa cells, leukocytes in blood from healthy individuals, smokers and non-smoker subjects with chronic periodontitis was evaluated[15]. The authors reported a higher percentage of hypomethylation of IL-8 in chronic periodontitis subjects (independent of smoking) in DNA of oral mucosal cells, no significant differences were observed in gingival and blood cells between different groups with regard to the methylation status. Another study evaluated the epigenetic changes in the promoter region of Prostaglandin synthase 2, the gene coding for cyclooxygenase (COX-2) in chronic periodontitis subjects. The results revealed a hypermethylation status of the gene and lower levels of COX-2 transcription in inflamed gingival biopsy[16].The DNA methylation status in the promoter region of IL-8 gene in oral and gingival epithelial cells of subjects with aggressive periodontitis revealed a hypomethylated status in subjects with generalized aggressive periodontitis[17].When the epigenetic modifications in the promoter region of interferon gamma (IFNG) gene in different stages of periodontal disease was evaluated, a significant hypomethylation and increased IFNG transcription in gingival biopsies were found in subjects with chronic periodontitis[18].

Another study which evaluated the methylation level of IFNG reported a similar methylation level of IFNG and IL-10 in chronic periodontitis gingival biopsies compared to healthy controls[19].A study evaluated the epigenetic changes in E- Cadherin and COX-2 genes in chronic periodontitis and non periodontitis subjects and breast cancer biopsies[20].The results showed a hypermethylation of E-Cadherin and COX-2 in similar proportions in breast cancer and chronic periodontitis subjects. It can be inferred from this study that epigenetic changes observed in the two genes in periodontitis subjects might be related to the irreversible destruction in the tissues similar to that observed in cancer. The significance of epigenetic alteration of cytokine levels is that it can influence the type of immune (Th) response and also regulate DNA methyl transferase expression in T cells, thereby play a significant role in regulation of immune response.

IV. Histone Modifications And Periodontitis

Very few studies are available on histone modifications in periodontitis[21,22].Results of the studies show that maintaining histone acetylation of genes related to osteoclastogenesis was found to be important for preventing bone loss in experimental periodontitis. Another study concluded that treatment with histone deacetylase inhibitor (HDACi) resulted in improved bone levels[23].Larsson etal[22] in their study demonstrated that different epigenetic modifications of DNA and histones influence IL-10 gene expression. The authors also found an association between lipopolysaccharide stimulation and histone modification. These alterations differed between IL-10 genotypes for the -1087 G/A polymorphism in the IL-10 gene promoter.

V. Epigenetic Influence Of Biofilm On Periodontitis Pathogenesis

The oral environment has diverse microbial profile. Plaque bacteria evokes host immune response in the gingival epithelium. The gingival epithelium utilizes multiple signalling pathways to regulate innate immune responses to various oral bacteria. But very little is understood as to how these bacteria alter the epithelial epigenetic status. Recent evidence has shown that bacteria belonging to the orange and red complex can cause epigenetic changes in the periodontal tissues[24].Epigenetic modifications may influence periodontal pathogens, because it was shown that an aberrant methylation mechanism can alter the virulence of A.actinomycetem comitans by decreasing the ability of the bacteria to invade oral epithelial cells[25]. A report of the bacterial influence on DNA methylation showed that bacterial infection with C.rectus promoted DNA hypermethylation of the IGF2 gene[26].Another study showed that stimulation with Treponema denticola on periodontal ligament cells showed a decrease in methylation of the MMP-2 promoter[27].However the MMP-2 promoter was found to be hypomethylated regardless of the presence of T.denticola. The authors suggested that the adherence and/or internalization of T.denticola may contribute to regulation of matrix degradation and bone resorption. Studies using culture supernatant from Porphyromonas gingivalis have shown high levels of butyric acid. These culture supernatants were reported to inhibit HDACs resulting in increased histone acetylation[28,29].

A study comparing the expression of epigenetic markers in gingival fibroblasts and keratinocytes showed that periodontal pathogens can induce cell specific changes[30].It was shown that P.Gingivalis lipopolysaccharide stimulation resulted in a decrease in mRNA transcription of DNMT1, DNMT3 and histone demethylases Jumonji domain containing 3 (JMJD3) in keratinocytes but not in gingival fibroblasts. However, RNA analysis from gingival biopsies from periodontitis and healthy sites showed no difference in gene expression from these target genes. The results of this study are consistent with that of Yin and Chung[31] . These results show that oral bacterial infection is associated with changes in the H3K4 methylation level and that the bacteria investigated induce different levels of the H3K4me3. Also, these bacteria show different epigenetic changes for the antimicrobial proteins human beta defensin 2 (hBD2) and CC chemokine ligand 20 (CCL20). Chung and Dale in their study reported a differential induction of downstream innate immune markers following exposure of gingival epithelial cells to different oral bacteria[32].The results showed that different signalling pathways were involved for different bacteria. Not only do bacteria influence the methylation level of hosts' genes, the bacterial DNA can also differ in methylation level, which influence their ability to induce the production of inflammatory cytokines. From the results of all these studies it can be inferred that periodontal pathogens can modulate the host defense through inducing epigenetic changes in transcription factor expression in gingival epithelial cells.

VI. Environmental Stressors As Epigenetic Modifiers

Environmental influences have been proposed to contribute to the progression of periodontitis. Alterations in DNA methylation status as a result of environmental stressors have been reported to begin before birth. Even though many epigenetic marks are potentially reversible, many epigenetic changes appear to persist throughout the cell lineage and life of the organism.

One of the important environmental risk factors for progression of periodontitis is smoking. Smoking causes long term hypo and hypermethylation changes in the DNA. Haffajee and Socransky in a study found that smokers had more severe form of periodontitis with more attachment loss and deeper pockets compared with former smokers and non-smokers. The severity of attachment loss was associated with age[33].The increase in attachment loss may be a result of epigenetic changes due to an increase in methylation in the collagen type 1 alpha 1 (COL1A1), a protein in the periodontal ligament seen in elderly individuals compared to younger individuals[34].These findings indicate that an age associated decrease in collagen in the periodontal ligament is caused by epigenetic modifications, thus showing that epigenetic modifications may constitute a risk factor for periodontitis. A study on the influence of smoking on global DNA methylation showed that smoking induces generalized alterations in DNA methylation across multiple tissues and organ systems[35].Oliveira etal evaluated the methylation status of Il-8 promoter gene in smokers and non-smokers with periodontitis and concluded that there was no significant difference between the two groups with regard to the methylation status. However, no studies on global methylation status have been conducted in smokers with periodontitis to assess the impact of smoking at an epigenetic level in subjects with periodontitis[36].

Intra uterine nutrition is said to influence epigenetic programming of the fetus. It has also been suggested that sensitivity to diet or to environmental toxins may vary among individuals due to pre-existing genetic variants that can challenge methyl metabolism and predispose individuals to epigenetic changes[37].The role of micronutrients in the pathogenesis of periodontitis has been elicited in previous studies. Several nutrient factors such as folate, vitamin B12 and vitamin A may result in changes in epigenetic modifications. It has been shown that folate deficiency during pregnancy leads to a lack of S-Adenosylmethionine a substrate required for the enzyme DNMT to methylate CpG residues during embryonic development[38].It has been proposed that sensitivity to diet or to environmental toxins may vary among individuals due to pre-existing genetic variants that can challenge methyl metabolism and predispose individuals to epigenetic changes. Other environmental stimuli that may potentially function as epigenetic modifiers include exposure to metals and aromatic hydrocarbons, fossil fuel emissions, contaminated drinking water, infection and cigarette smoke.

It has been shown that epigenetic changes increase during life and that age itself is a risk factor for epigenetic changes. Fraga etal[39] in a study on monozygotic twins demonstrated that 35% of the monozygotic twins differed in both methylation and histone pattern.These differences increased as the twins grew older, together and by differences in disease history. Also, a comparison of epigenetic patterns in 3 year old twins with those of 50 year old twins showed large difference in gene expression patterns between the age groups. There was marked difference in both DNA methylation and histone acetylation in the lymphocytes in older twins. These findings suggest that epigenetic changes increase during life and that age itself is a risk factor for epigenetic changes. These findings also indicate a potential link between changes in epigenetics and chronic inflammation such as periodontitis and raises the question whether older periodontitis patients have the same epigenome in the inflammatory lesion as younger patients.

VII. Epigenetic Basis For Immune Regulation In Periodontitis Pathogenesis

The immune response is not only regulated by genetic factors, but there is a second level of regulation related to the chromatin status of the DNA. It has been suggested that an individual's genotype and immunological defence against bacteria determine the susceptibility for developing chronic periodontitis[40].In another study on patients with systemic lupus erythematosis (SLE), hypomethylation was found in the promoter region of the IL-4 and IL-6 genes. There was also a concomitant increase in gene expression in T cells in these patients[41].The authors suggested that the increase in cytokine expression and pathological T cells might be a result of spontaneous demethylation in certain cytokines. Epigenetic alterations have also been found in rheumatoid arthritis. These findings indicate that chronic inflammatory processes may be the result of a decrease in histone deacetylase activity as well as changes in methylation which subsequently leads to an increase in gene expression of inflammatory factors.

Little is known of the role of host inflammation on modification of epigenetic patterns but the activation of the immune response involving potential epigenetic changes has been suggested[42].Inflammatory signals that activate nuclear factor kappa B has been shown to alter the histone methylation pattern and activate gene expression. Thus it can be said that inflammation has some potential to modify chromatin structure via histone structure; however, the role of inflammation in modulating CpG methylation patterns which are more likely to be conserved following cell replication remains unclear. Recent studies report that the loss of epigenetic control over this complex process contributes to autoimmune disease[43].

A pilot study was conducted to assess whether oral biofilm could epigenetically modify local adjacent periodontal tissues. The results of the study showed the role of bacterial infection and chronic inflammation as a potential stimulus for altering local periodontal tissue DNA methylation pattern. This provides a fertile area for further investigation.

MicroRNAs (miRNAs) are non coding RNAs that inhibit gene expression by binding to mRNA, by a sequence pairing homology to regulate and fine tune gene expression. miRNA expression is said to be altered in response to bacterial products but also inflammatory cytokines can influence the regulation of miRNA production[44].Another class of non coding RNAs, the long non-coding RNAs (IncRNAs) bind to chromatin regulatory proteins there by controlling access or inhibition of proteins binding to enhancer regions in the DNA. One function of these RNAs in the immune response has been suggested to involve the regulation of host response, including innate immunity. A recent report identified a new non-coding RNA that are linked to epigenetic mechanisms[45].

VIII. Periodontitis as a risk for systemic diseases – an epigenetic link

Periodontitis has been proposed as a comorbidity factor for several systemic conditions. Very few studies have evaluated the epigenetic changes in systemically compromised individuals with periodontitis. Perri etal studied the expression profile of micro-RNA in obese individuals with and without periodontitis[46]. In obese individuals with periodontal disease, different mi-RNAs were upregulated. These micro-RNAs are said to be involved in the regulation of genes coding for cytokines, collagen, chemokines and some important lipid mediators. The significance of variation in these levels need to be evaluated further to clearly understand the significance of these findings and their impact on periodontitis as a risk for systemic diseases. Bobetsis etal in a study showed that periodontal infection could lead to placental-fetal exposure and when coupled with a fetal inflammatory response lead to preterm delivery[47].Epigenetic modifications represent a fertile field of research for establishing a link between focal infections such as periodontitis and systemic diseases.

IX. Clinical Applications Of Epigenetic Therapy

Since it has been suggested in the literature that epigenetic changes may influence disease susceptibility and progression, one may speculate that this could open up new treatment models reversing these changes. Histone deacetylase inhibitors have been used for the treatment of chronic inflammatory diseases involving bone. Limited research has been done using HDAC or DNA methylation inhibitors as treatment for periodontitis or oral inflammation. Treatment using a HDAC inhibitor, 1179.4b showed reduced alveolar bone loss and less osteoclasts/mm^2 in mice with P.gingivalis induced periodontitis compared to untreated mice. However, no influence was found on the level of inflammation which indicated a direct effect of this HDACi on bone regeneration rather than an indirect effect targeting inflammation[48].

A recent study analysed the function of BET inhibitor JQ1 in inhibiting inflammatory response and alveolar bone loss in experimental periodontitis. The findings suggested that JQ1 may be a potential treatment model for periodontitis[49].The results of the above studies represent preliminary work on epigenetics as part of host modulation therapy for the management of periodontitis. Another study reported the potential use of histone deacetylase inhibitor, sodium butyrate in inducing the differentiation of periodontal ligament fibroblasts into osteoblasts. The authors concluded that this histone deacetylase inhibitor was a potential therapeutic agent for periodontal regeneration[50].

X. Conclusion

Epigenetic modifications caused by smoking, diet, age, bacteria and inflammation can affect oral health and contribute to disease susceptibility. Epigenetics could be the possible link between the genome and the environment and perhaps provide new data on patient susceptibility. Biofilm induced epigenetic patterns may influence local tissue metabolism there by altering the microbial ecology and local healing response of periodontal tissues. Analysis of epigenetic modifications in the oral mucosa is important to gain knowledge of the effect of these mechanisms on oral health. Investigation into epigenetic patterns in the oral mucosa may provide new treatment models not only for periodontitis but also for other oral diseases. Future research have to be undertaken to investigate and assess the extent to which environmental factors such as smoking and diet influence epigenetic changes that can predispose to periodontal disease.

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