

Epigenetics - A Window Towards Personalized Periodontal Therapy

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Abstract: Periodontitis is characterized by infection and inflammation of tooth supporting tissues. Even though pathogenic microorganisms are the primary etiological factor, the progression is modified by genetic factors of the individual. Many environmental factors such as smoking, inflammation, malnutrition are identified as risk factors. Epigenetic modifications occur in response to environmental changes which can affect gene expression and is not mediated by mutations. Recent research using genome wide analysis highlights that genetic variations are associated with an increased risk of periodontitis. Epigenetic drugs may provide an opportunity to develop tailor made therapies and enhance the effects of combination therapy.

Keywords: dna methylation , environmental factors, epigenetics, genetics, periodontitis

I. Introduction

Periodontal disease is a chronic inflammatory disease of multifactorial etiology. Microorganisms associated with dental plaque were thought to be the sole factor for periodontal destruction until 1970's. Bacteria are essential but alone will not produce the disease. Imbalance in host response dictates susceptibility to diseases. Risk factors play an important role in the development of periodontal diseases. Many environmental factors such as diet, smoking, inflammation, chemicals, drugs and age may affect gene regulation, which leads to epigenetic modification in the genome (Fig. 1). Epigenetic information can be inherited by modification in histone[1] by DNA methylation [2] which are enzymatically copied. Host-microbial interactions along with environmental factors are major determinants for the development of periodontal diseases and hence, for the relationship between genotype and phenotype [3].

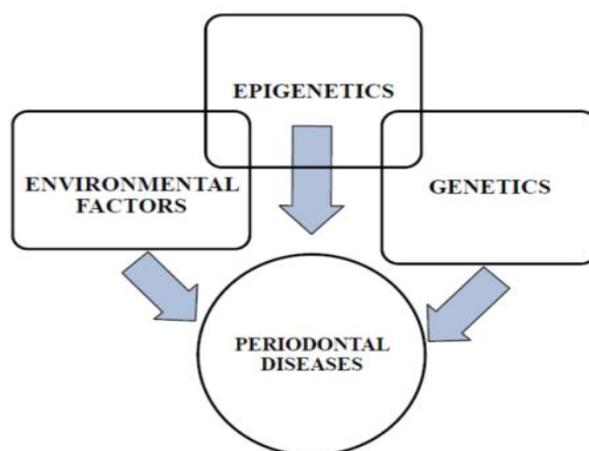


Figure 1 Influence of Environmental factors, Genetics and Epigenetics in Periodontal diseases

The term Epigenetics means 'upon the genome'. Epigenetics is the link between environment and phenotype. Epigenetic modifications involve DNA methylation, Histone modification, and gene regulation by non-coding RNAs. Epigenetic modifications lead to the activation and inactivation of a gene. These changes can result in the development of cancer and autoimmune or inflammatory diseases, including periodontitis [4]. Unlike genetic mutations, epigenetic changes can be reversed. Epigenetic alterations and its associated diseases do not respond to traditional therapies. Drugs that reverse epigenetic modifications are known as Epi-drugs. Personalized medicine can be visualised as a tailored therapy based on the interactions between genetics, clinical and environmental factors affecting that individual [3].

II. History Of Epigenetics

Conrad. H. Waddington in 1942 coined the word 'Epigenetics'. Holliday R in 1994 defined Epigenetics as: i) The study of the changes in gene expression which occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression, ii) Nuclear inheritance which is not based on changes in DNA [5]. In 2008, a consensual definition of epigenetics was established as a 'stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence' [6]. Epigenetic changes occur more frequently than genetic changes. The epigenetic changes can be reversed by treatment with pharmacological agents.

III. How Do Epigenetic Modifications Occur?

Diet or environmental toxins, exposures to metals, aromatic hydrocarbons (e.g., benzopyrene) found in occupational chemicals, contaminated drinking water, cigarette smoke, fossil fuel emissions and infection predispose to epigenetic changes [7,8]. The role of environmental factors and ageing was confirmed by a pioneer study in epigenomes of monozygotic twins which highlighted that the twins were identical in their early years of life, however later in life the older twins had different lifestyles and spent less time together had significantly diverged epigenomes [9]. Epigenetic modifications turn genes on or off which prevents or allows the gene to make a protein resulting in diseases. Modifications in gene expression are controlled by mechanisms like DNA methylation, Histone modification, non-coding RNAs and chromatin remodelling .

3.1. DNA METHYLATION

DNA methylation occurs in the CpG dinucleotides in the promoter region of the gene [10]. CpG means "—C—phosphate—G—". It occurs mainly on the fifth carbon of the cytosine base, forming 5-methylcytosine or 5-methylcytidine (5-mC) mediated by a group of enzymes called DNA methyltransferases (DNMTs), - DNMT1, DNMT3a and DNMT3b. DNMT3a and DNMT3b are de novo methyltransferases that are able to methylate previously unmethylated CpG dinucleotides. Hypermethylation of promoter region of genes leads to loss of gene expression. Hypomethylation is associated with gene expression.

3.2. HISTONE MODIFICATION

Histones can be modified by the addition of acetyl, methyl, or phosphate groups [11]. Histones can be displaced by chromatin remodelling complexes, thereby exposing underlying DNA sequences to polymerases and other enzymes [12]. Histone proteins can be extensively modified with a wide array of posttranslational modifications. Histone acetylation is the acetylation of histone tails by HAT's (histone acetyl transferases) correlates with transcriptional activity in many genes. Histone deacetylation (the removal of acetyl groups from nucleosomes, by HDAC's (histone deacetylases) causes repression of gene expression. There are two main classes of HDACs, Class I and Class II. Class I includes HDACs 1, 2, 3 and 8, which are found in the nucleus. Class II includes HDACs 4, 5, 7 and 9 that belong to class II a HDACs and HDACs 6 and 10 belonging to class II b HDACs, which are able to shuttle between the nucleus and the cytoplasm [13].

3.3. NON CODING RNA

Non protein coding RNAs (NcRNAs) contribute to epigenetic regulation. NcRNA molecules inhibit gene expression by interactions with the nascent RNA molecule, DNA itself or participating in recruitment of chromatin modifiers [14].

3.4. CHROMATIN REMODELING

Chromatin remodeling refers to changes in chromatin location and structure. This in turn will give rise to loss of tightened chromatin in nucleosome joints to expose cis-acting elements in the gene promoter thereby providing a chance of combination with trans-acting factors [15].

IV. Environmental Factors

Epigenetic changes are influenced by environmental factors and are reversible unlike genetic changes in DNA. Disease susceptibility and differences in response to treatment could be contributed to epigenetic modifications caused by diet, smoking, bacteria and inflammation. The role of environmental factors has been confirmed by studies done in twin models. Age is considered as a risk factor for epigenetic changes and these changes increase as the age advances. Difference in methylation and histone pattern was demonstrated in 35% of the monozygotic twins in a study done by Fraga et al. Nutritional factors like vitamin A, D, B12 and folate may cause epigenetic modifications thereby influencing disease progression. Smoking causes long lasting epigenetic changes in the DNA. These are found in current and former smokers. Chronic inflammatory diseases may be due to decrease in histone deacetylase activity /changes in methylation which leads to altered gene expression [16].

4.1.Bacteria

Evidences suggested an association between oral bacteria and genetics. In patients with severe periodontitis, polymorphisms found in Interleukin-6(IL-6)genes were found to be consistent with the presence of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. Epigenetic modifications may influence periodontal pathogens, which inturn cause epigenetic changes in the host, further enhancing disease progression [16].

V. Role Of Epigenetics In Periodontal Diseases

Inflammatory response initiates an immune response that involves both innate and adaptive immunity. Epigenetic regulation of gene expression patterns upregulate proinflammatory cytokines and other signalling molecules to activate a full response from immune cells, while simultaneously down regulate anti-inflammatory cytokines. The cytokine genes have been suggested as targets of multiple epigenetic events. IL-1, IL-2, IL-6, IL-8, IL-10, IL-12 gene [17], TNF α and IFN γ may be regulated by epigenetic mechanisms. TLR-2 and TLR-4, associated with an increased proinflammatory response, are regulated by DNA methylation. Epigenetic mechanisms play a key role in the initiation and progression of inflammation. TLR4-dependent reprogramming of inflammatory genes is mediated by two levels of regulation [18]. Inflammatory signals which activate NF- κ B have shown to alter histone methylation patterns and activate gene expression [19]. Thus, inflammation has some potential to alter chromatin structure via histone structure. Evidences suggest that bacteria play an important role in altering cellular DNA methylation status [20] and that environment, aging and stress are involved in epigenetic modification that modify gene expression and disease expression. Epigenetic modifications provide a plausible link between the environment and alterations in gene expression that might lead to disease phenotypes.

VI. Evidence For The Role Of Epigenetics In Periodontal Disease

The role of epigenetics in periodontal disease has been investigated with great interest over the last few years and is documented in the literature. Epigenetic remodelling events are critical for inducing inflammatory response. Zhang et.al reported a lower methylation level of CpG sites and an increase in IFN- γ transcription in gingival biopsies of chronic periodontitis sites compared with periodontally healthy controls [21].

Viana et.al evaluated DNA methylation in IFN- γ , IL-10in gingival biopsies of periodontitis and non periodontitis patients .The results showed methylation level of IFN- γ and IL-10 was similar in both groups. Most samples were positive for IFN- γ methylation. Of samples in the periodontitis group, 11% were unmethylated, whereas no unmethylated samples were found for IL-10 [22].

Association of epigenetic modifications in the TNF promoter in human gingival biopsies from different stages of periodontal diseases was investigated by Zhang et.al . DNA methylation may be an important regulatory mechanism in controlling TNF- α transcriptional expression in periodontal disease [23]. Another study conducted by Stefani et.al evaluated DNA methylation of IL-6 in gingival biopsies of chronic periodontitis patients and controls, reported that there is no difference in methylation of IL-6 [24].

The progression to periodontitis from gingivitis is characterized by a transition from Th1 to Th2 subsets [25]. During development and differentiation of naïve T cells into various lineages, changes in the chromatin structure occur through epigenetic mechanisms like DNA methylation and histone modification. Cytokine genes which define the lineage specificity include IFN γ gene for Th1 cell lineage, IL-4 gene for Th2 cell lineage, and IL-17 gene for Th17 cells [26]. The expression of one cytokine gene and the permanent silencing of the other are orchestrated using epigenetic mechanisms [27]. Loo et.al reported a similar proportion of hypermethylation of E-Cadherin and COX-2 expression in chronic periodontitis and breast cancer biopsies compared to non periodontitis subjects. The epigenetic changes observed in periodontitis subjects are attributed to the irreversible destruction in the tissue [28].

Yin and Chung reported that the presence of bacteria caused epigenetic modifications in gingival epithelia and exposure to different oral bacteria results in differential methylation profile [29]. *P.gingivalis* significantly decreased the tri-methylation of histone H3 K4 protein expression, but *F. nucleatum* did not, which indicates that *P.gingivalis* could suppress the activation of transcription.

VII. Tools To Unravel The Epigenetic Mechanisms

A variety of methods are applied for the study of epigenetics to elucidate the molecular mysteries in epigenetic modification. Epigenetic research use mouse as a model organism to study early development.

The different approaches include:

- a) Sodium bisulfite modification
- b) Sequence-specific enzyme digestion,
- c) Methylated DNA Immunoprecipitation,
- d) Chromatin immunoprecipitation(ChIP),
- e) RIP(RNA Immunoprecipitation).

Recent advances in the field of epigenetic approaches will allow high accuracy mapping of the methylation state which in turn may help in the identification of biomarkers.

VIII. An Update On Epigenetic Drugs

Epidrugs are defined as “drugs that activate or inhibit disease-associated epigenetic proteins for ameliorating, curing, or preventing the disease” [30]. DNA methylation inhibitors (DNMTi) and Histone deacetylase inhibitors (HDACi) are used for the reversal of the epigenetic alterations inside the diseased genome. DNMTi include 5-aza-2-deoxycytidine, zebularine, Procaine, procainamide and MG 98 and HDACi include TrichostatinA, Pyroxamide, Trapoxin A and B and MS 275.

Clinical trials have been undergoing in this field. U.S. Food and Drug Administration has approved Two DNMT inhibitors and two HDAC inhibitors. 5-azacytidine and 5-aza-2'-deoxycytidine were approved for the treatment of higher-risk myelodysplastic syndrome. Vorinostat, Romidepsin (HDAC inhibitors) were approved for the treatment of cutaneous T cell lymphoma (CTCL) patients [31].

Treatment by HDAC inhibitors efficiently suppressed periodontal bone loss in a mouse model of periodontitis [13]. Treatment with novel HDAC inhibitors, such as 1179.4b (class I, II HDACi) and MS-275 (class I HDACi), on *P.gingivalis*-inoculated mice resulted in significantly reduced *P.gingivalis* induced bone loss with 1179.4b, whereas MS-275 had no significant effect, indicating that maintenance of acetylation is crucial to preventing bone loss and no influence was found on the level of inflammation. HDACi prevent bone loss and demonstrates that the novel HDACi, 1179.4b reduces alveolar bone loss in an in vivo model of periodontitis [13].

Kim et al., 2002 [32] reported on the use of histone deacetylase inhibitor (sodium butyrate) in inducing the differentiation of periodontal ligament fibroblasts into osteoblasts and concluded that sodium butyrate was a potential therapeutic agent for periodontal regeneration. Treatment with 5-aza resulted in an increase in TNF- α mRNA expression in periodontitis patients [22]. Combinations of drugs that inhibit DNMTs as well as HDACs may result in synergistic effects.

IX. Clinical Significance And Personalized Periodontal Therapy

Every person has a unique variation of the human genome. Personalized medicine can be foreseen as a tailored therapy based on the interactions between genetic, clinical and environmental factors affecting that individual. Recent advances in epigenomic approaches allow mapping of the methylation state in the genome, which may help in the identification of epigenetic biomarkers [33].

Individualized periodontal therapy is the upcoming concept of medical treatment for an enhanced clinical outcome. Genomic approaches are the useful tools that bridge epigenetics and personalized medicine. Patients with epigenetic alterations and associated disorders do not respond to conventional therapy. Therefore, drugs used for personalized medicine can be used to manage these disorders based on an individual's personal genomic profile.

X. Conclusion

Periodontal diseases are multifactorial where genetics and environmental factors interact with each other to determine the susceptibility of the host to inflammation. Epigenetic modifications are responsible for the differential expression of our genetic material. Epigenetic modifications are potentially reversible. Identifying epigenetic patterns associated with the development of periodontitis may also improve individualized approaches that can be used to personalize treatment plans. Individualized and improvised diagnostic tools for earlier detection and ways to halt the disease helps in regeneration and reconstruction of the lost periodontal attachment apparatus with the biology based approaches.

Acknowledgements

I would like to express my sincere gratitude to Dr. Seba Abraham, Professor & HOD and Dr. Ambili R, Professor for their continuous support and guidance throughout the preparation of this manuscript .

Conflict of interest

No conflict of interest and no financial interest as well.

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