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Epigenetics in periodontics – A brief review

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Abstract

Periodontal disease is a chronic inflammatory state resulting in the destruction of periodontal structures in susceptible individuals due to immune response to various etiological factors. The involvement of specific subjects and the site-specific nature of the disease and the variations in the response of the treatment suggests its strong association with genetics and epigenetic changes. The term epigenetics relates to changes in gene expression that are not encoded in the DNA sequence itself and include chemical alterations of DNA and its associated proteins. The purpose of this review article is to highlight the basic role of epigenetics in periodontal disease.

Keywords: DNA methylation, deacytylase, Epigenetics, histone modification

Introduction

Periodontitis is a multifactorial infection characterized by inflammation and destruction of tooth supporting tissues, due to the response of a susceptible host to bacterial challenge. Although the definite mechanism is still unclear, a contributing factor is the outgrowth of microorganisms in the oral cavity which triggers the host immunity[1]. Various extrinsic and intrinsic factors such as genetics and epigenetics are also considered as an aetiologic factor[1]. Apart from that many environmental factors such as diet, smoking, inflammation, chemicals, drugs, and age may affect gene regulation, which leads to epigenetic modification in the genome[2].

In Greek, prefix 'epi' in epigenetics means 'on the top of' or 'in addition to' genetics[1]. Epigenetics is described as changes in pattern of gene expression, which do not involve changes in the DNA sequence[3]. Although they play an important role during development and pathological disease of the oral cavity, the use of epigenetics is still at an early stage in dentistry. Epigenetic changes unlike random mutations, involve modifications which are flexible and responsive to environmental challenges.[4]. Epigenetics is defined as a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence (Cold Spring Harbor meeting, 2008)[6]. This review article attempts to

brief out the concept of epigenetics and its role in periodontal disease.

History

The history of epigenetics evolved with the study of evolution and development[5]. Dr. C.H Waddington, an embryologist, put for a radical idea of its era in early 1940's. He introduced the concept of genes and their regulation via an epigenetic landscape, as controlling cell fate and how cells specialize[6].He coined the term 'Epigenetics' in the year 1942 which referred to the study of causal mechanisms by which "the genes of the genotype bring about phenotypic effects"[6].

Mechanism of epigenetics

The mechanisms which bring about epigenetic modifications include chemical modification of DNA (e.g; as induced by DNA methylation and histone modification) and those resulting from the action of small noncoding RNAs. These alterations allow the single genome to adapt its transcriptional repertoire to the ever changing environmental conditions. The main mechanisms include,

- DNA Methylation
- Histone modification
- Non coding RNA

DNA methylation

This refers to the addition of a methyl group (_CH3) covalently to the base cytosine in the dinucleotide 5´-CpG-3.[7]One theory on the evolution of DNA methylation is that it evolved as a host defence mechanism to silence foreign DNA such as viral sequences, replicated transposable elements and other repetitive sequences.

- HYPERMETHYLATION of promoter region of genes is associated with transcriptional silencing of gene thereby leading to loss of gene expression. [8]
- HYPOMETHYLATION of promoter region of genes is associated with transcriptional activation of gene thereby leading to gene expression. [8]

Histone modification

The nucleosome consists of a DNA segment and eight core histones (H1, H2, H3, and H4) (Campos and Reinberg, 2009).[1] Histone proteins can be modified by various post-translational modifications to alter the DNA-histone interaction and change the conformation of chromatin. It either condenses or relaxes the chromatin. The modifications take place at N-terminal tails of the protein. Most modifications are found in the H3 and H4 histones and these modifications include methylation, acetylation (fig 1)[28], phosphorylation, ubiquitinylation, simulation, citrullination and ADP-ribosylation (Wysocka J, 2006).[9]

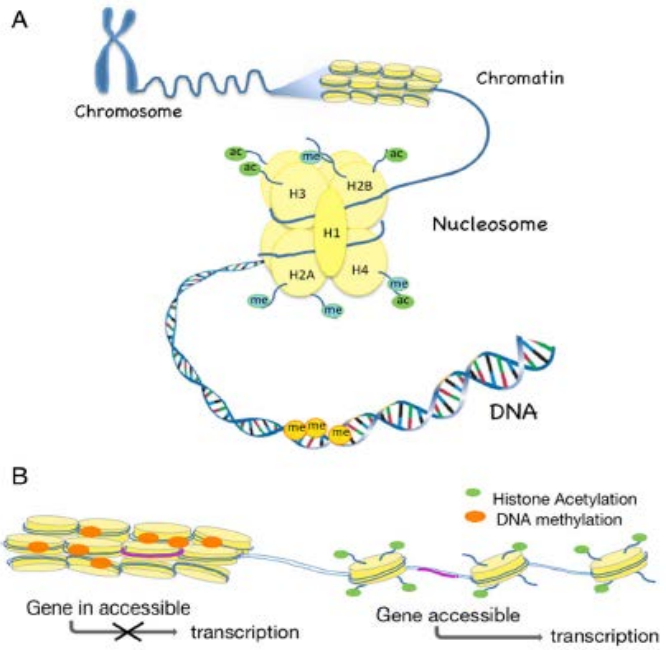

Fig 1. illustrates Schematic overview of the structure of the chromatin (a) and the epigenetic modifications of DNA methylation and histone modifications and their influence on chromatin formation and gene expression (b). Ac acetylation, Me methylation.

Non coding RNA

A non-coding RNA (ncRNA) is a functional RNA molecule that is transcribed from DNA but not translated into proteins. Although it has been generally assumed that

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most genetic information is transacted by proteins, recent evidence suggests that the majority of the genomes of mammals and other complex organisms is in fact transcribed into ncRNAs, many of which are alternatively spliced and/or processed into smaller products.[10]

Epigenetic related ncRNA's include miRNA, siRNA, piRNA and lncRA.

Factors effecting epigenetics

Epigenetics can be induced or altered by various factors in the environment (fig 2) that modulate the gene expression and affect various gene functions. Therefore, it produces link between the inherited genome and the environment[1]. Several lifestyle factors have been identified that might modify epigenetic patterns, such as diet, obesity, physical activity, tobacco smoking, alcohol consumption, environmental pollutants, psychological stress, working habits. Inividual genetic background and environmental factors are interwined to lifestyle in determining the health status of individuals.[11]

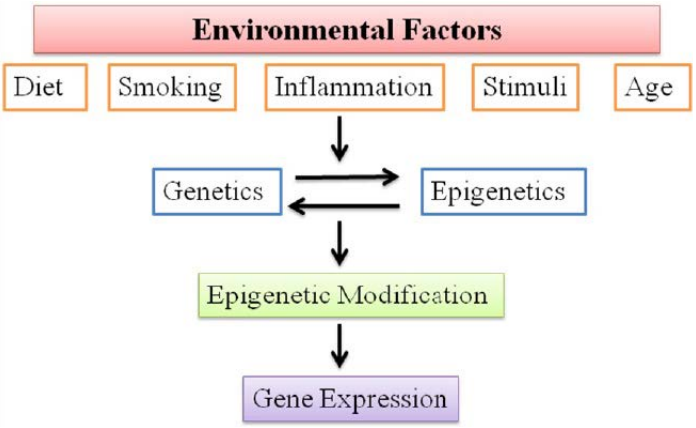

Fig 2: Various factors influencing epigenetic modification.[1]

Role of epigenetics in periodontitis

The gene expression is mainly determined by epigenetic events such as remodeling of chromatin and selective activation or inactivation of genes[8,12]. The exact role of epigenetic changes on bacteria-induced host inflammatory response is still under research. Patients with the same clinical features respond in a different manner to the treatment, suggesting involvement of genetic as well as epigenetic factors[8,13].

It is already well established that inflammation in the periodontium is initiated by the microbial biofilm[14,15] and its persistent perturbation leads to constant cellular death and turnover leading to tissue regeneration process that could invoke specific epigenetic modifications resulting in a different phenotype.[14]

Studies have also shown that the factors which regulate differentiation such as extrinsic environmental factors, growth factors, and hormones can cause epigenetic modifications[14,16] Also lifestyle of smoking, lack of nutrition, lack of exercise, and use of drugs strongly influences the epigenetic pattern and predisposes to most conditions that lead to human disease.[14,17] Hence it can be speculated that this process can be interlinked with periodontal disease.

Chronic periodontitis invokes a high turnover of cells in the gingiva such as epithelial cells, fibroblasts, and bone-forming cells necessary to replace cells lost due to cellular apoptosis.[14]

Any defect in these proliferating cells can lead to an aberrant cellular response that in turn leads to a variation in phenotype. High levels of cellular turnover and change in microbiota in the host may create discriminatory pressure in certain lesions leading to patches of epigenetic reprogramming.[14]

In fact, periodontal disease is characterized by its variation in the disease susceptibility in an individual along with site variations where in certain areas showing the majority of the disease in any given individual and other sites are relatively disease-free. There is a persistence of this disease susceptibility and progression at the site level. [14]

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The traditional explanation for site prediction is related to site plaque accumulation but even after treatment and with optimal hygiene these sites remain susceptible and thus it is hypothesized that they are epigenetically altered in their inflammatory responsiveness. To help explain this phenomenon, the term “periodontal mosaicism” was coined for this variegated phenotype in periodontitis and it is defined as epigenetic variation in the gingiva leading to variation in disease susceptibility.

Treponema denticola was found to induce hypomethylation of the MMP2 promoter and a chronic activation of pro-MMP2 in PDL cells[28,29]. This indicates that T. denticola through epigenetic mechanisms play a role in activation and enhancement of the loss of supporting tissue seen in periodontitis.

In summary, these studies add to the knowledge on how dysbiosis epigenetically influence molecular signaling within the host immune response as well as on how the local microenvironment around cells can play a part in regulating activation and/or production of signaling molecules that further influence tissue degradation and uphold a chronic inflammation.

Although epigenetic studies in periodontitis are limited, several existing studies suggest that these cells have the ability to respond to environmental factors in attaining different phenotypes.[14]

Epigenetic imprint of biofilm on periodontitis pathogenesis

The plaque bacteria evoke host immune response in the gingival epithelium. Gingival epithelia utilize multiple signalling pathways to regulate innate immune responses to various oral bacteria, but little is understood about how these bacteria alter the epithelial epigenetic status.[8] Recent evidence has shown, bacteria belonging to the orange and red complex can cause epigenetic changes in the periodontal tissues.[8,18]

A recent study by Yin and Chung [8,19] provides a new insight into the bacteria-specific innate immune responses via epigenetic regulation. The authors reported that the presence of bacteria results in epigenetic modifications in gingival epithelium and exposure to different oral bacteria results in differential methylation profile.

In the study, stimulation of gingival epithelial cells (GECs) with Fusobacterium nucleatum resulted in hypermethylation of mucosa associated lymphoid tissue lymphoma translocation gene 1 (MALT1) and thereby a lack of nuclear factor kappa B production by GEC.

On the other hand, stimulation of GEC with Porphyromonas gingivalis resulted in hypomethylation of ZNF287 a DNA binding protein believed to be involved in transcriptional regulation.

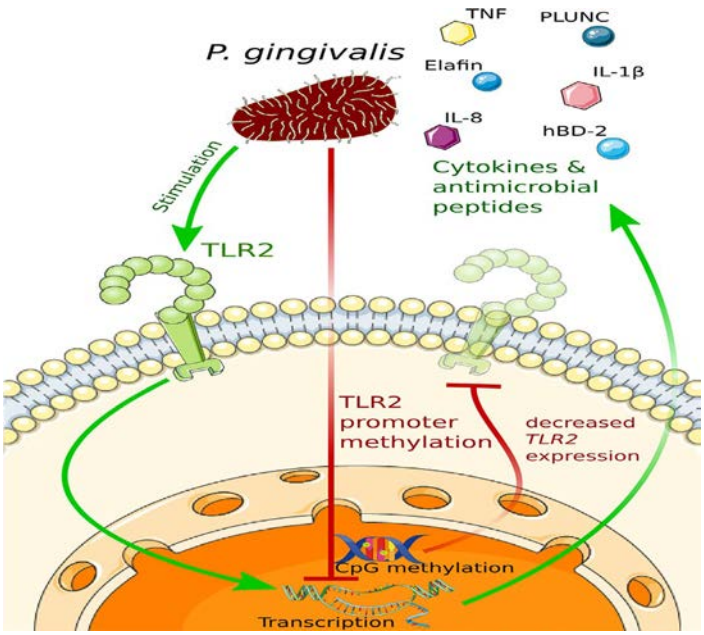

Fig 3: Model of epigenetic regulation of TLR2 expression in GECs. (This figure was created using images from Servier Medical Art (http://smart.servier.com). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License)

Cell responses to P. gingivalis are predominantly mediated by engagement of TLR2(fig 3)[32]. Under physiological

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conditions, P. gingivalis-induced activation of TLR2 stimulates the production of inflammatory cytokines, chemokines and antimicrobial peptides that promote pathogen elimination by the immune system. Chronic exposure to P. gingivalis, induces increased TLR2 promoter methylation in GECs, which was also observed in cells from a subgroup of periodontitis patients[30].TLR2 promoter hypermethylation is associated with reduced TLR2 expression and diminished production of inflammatory mediators and antimicrobial peptides normally induced by the pathogen.[30]

DNA methylation and periodontitis

Changes in DNA methyltransferase levels have been observed in cells exposed to whole bacteria lysate or to purified lipopolysaccharide.[4] Other in vitro studies demonstrate that different epithelial cell lineages, such as oral keratinocytes, immortalized human keratinocytes (HaCaT cells), and gingival epithelial cells, when stimulated by Fusobacterium nucleatum, Porphyromonas gingivalis, or purified lipopolysaccharide, show a significant reduction in the levels of DNA methyltransferase-1 expressed.[4] Zhang et al,[20] evaluated the presence of epigenetic modifications in the promoter region of interferon gamma (IFNG) gene in different stages of periodontal disease and the authors reported a significant hypomethylation and increased IFNG transcription in gingival biopsies from chronic periodontitis sites.

In a recent study, the DNA methylation pattern of TLR2 was investigated in human gingival epithelial cells(HGECs). It was shown that growing epithelial cells in the presence of P. gingivalis induced DNA methylation. A similar finding was also found in the gingiva of mice treated with P. gingivalis[30]. An activation of TLRs by periodontal pathogen not only induced histone acetylation in oral epithelial cells but also activation of transcription factor Nuclear factor-κb (NFκB)[22]. NFκB is a transcription factor that activate and co-ordinates the innate immunity as well as participate in osteoclast differentiation and induction of Matrix metalloproteinases (MMPs) and adhesion molecules[22]. Interestingly, DNA methylation CpG sites around NFκB binding site in the TLR2 promoter region have been identified. It may be speculated that alterations in DNA methylation can affect the binding of NFκB to the promoter hence, influencing activation and regulation of TLR expression[31].

Histone modifications and periodontitis

Very few studies are available on histone modifications in periodontitis. 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.[21] Martins et al found that P. gingivalis, lipopolysaccharide and heat inactivated F. Nucleatum induce abrupt, but short-lived, acetylation of histone H3 in oral epithelial cells.[22]Interestingly, Escherichia coli lipopolysaccharide resulted in a delayed, but powerful, induction of histone acetylation compared with that induced by P. gingivalis and F. nucleatum. The impact of histone modifications in periodontal diseases is unclear but nuclear factor-kappa B signalling appears to play a key role in connecting histone modifications to disease progression by the orchestration of inflammatory responses.[4]

Non-coding RNA

One of the most important miRNAs in periodontitis is miR-155, which acts at different stages in the host response. This miRNA is able to downregulate NF-κB signalling pathway, as well as to promote cell differentiation [23,24]. It also mediates the response to infection by type I interferon production, a mechanism possibly connected to aggressive periodontitis, increasing inflammation and periodontal tissue destruction. [23,25]

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Potential epigenetic pathways involved in the pathogenesis of periodontitis

As depicted in fig 4, the periodontopathic bacteria stimulates the gingival epithelial cells which results in epigenetic changes mainly by triggering pathogen-associated molecular pattern signaling pathways, immune regulatory mechanisms, and cytokine production variability which may contribute significantly to periodontitis pathogenesis.[8]

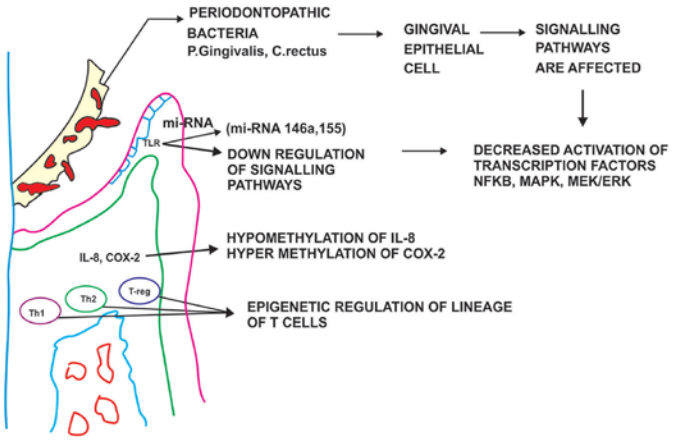

Fig. 4: illustrates epigenetic pathways involved in periodontitis.

Clinical application of epigenetics in the field of periodontitis

Periodontal Diagnostics - The Epigenetic Implications

Currently, the diagnosis of chronic periodontitis relies on clinical examination estimating pocket depths, clinical attachment levels, bleeding, plaque index and the use of x-rays or other radiographic methods. The emerging understanding of epigenetics can be utilized for novel methodologies to diagnose and assess the risk of a patient and tailor an individualized medicine considering the biofilm characteristics of an individual .[26] Papapanouet al[26,27]consistently demonstrated that, even among periodontal pockets with similar clinical characteristics, the subgingival colonization patterns still influence the transcriptome of the adjacent gingival tissues. The phenotype of the periodontal pocket is dependent on its bacterial content, and so is the susceptibility for further breakdown, and the treatment response. Such observations strongly suggest the utility of microbial diagnostics in the decision-making and therapeutic management of patients with periodontitis.[26]

Epigenetic in periodontal therapeutics

Currently US Food and Drug Administration (FDA) approved epigenetic molecules being investigated in treatment of cancer. Also, there are reports of using these molecules in inflammatory diseases. [28] In a recent review on the influence of HDAC inhibitors (HDACi) on bone remodelling, it was reported that HDACi influence osteoclast differentiation, maturation and activity. In addition, it has been found that HDAC inhibitors suppress bone loss in rheumatoid arthritis (RA) as well as in periodontitis and has therefore been suggested as potential treatment models for these diseases.

Many epigenetic drugs also have been discovered in the recently that can effectively reverse the causal epigenetic aberrations that occur in the disease condition, leading to the restoration of a normal epigenome. Histone deacetylase inhibitors have been used for management of chronic inflammatory diseases involving bone. The deacetylase inhibitors help in promoting osteoblast maturation and suppressing resorption of bone by osteoclasts.[8] The various epigenetic markers that have been targeted with inhibitors to modulate epigenetic influences are summarized in Table 1 .[8]

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Epigenetic targets	Inhibitors
DNA methyl transferase	5-Azacytidine Hydralazine 5-Aza-2/-deoxycytidine
Histone deacetylase	Valproic acid Suberoylanilidehydroxamic acid Entinostat Sodium butyrate
Histone demethylase	Monoamine oxidase inhibitor-Tranylcypromine

Table 1: Epigenetic marks for therapeutic intervention and their inhibitors

Although epigenetic therapy seems to be useful in treating some epigenetic disorders, adverse effects have been reported for many epigenetic drugs. As these drugs could get incorporated into DNA, the issues regarding their safety and potential toxicity on normal host cells were raised, but their action was reserved only to act on dividing cells led to their use, with minimal effects on slowly dividing normal cells.[26]

Tissue engineering

Scaffolds with nanostructured topography have been suggested as a potential tool to improve periodontal tissue engineering. Even though research on how surface topography and material energy affect the epigenome is in its early stage, the current knowledge indicates an interesting possibility to use materials and nanotechnology to promote tissue regeneration and cellular functions through epigenetics. Silica is a material that has been approved by the FDA as a delivery vehicle for DNA methylation inhibitor 5-aza. To improve tissue engineering, biochemical molecules are used to induce a specific function in cells or to induce differentiation of cells towards a specific cell type.[28]

Conclusion

Periodontitis is a complex disease with a mosaic of cells, cytokines, and signalling pathways involved in the activation and regulation of the immune response and tissue destruction. Knowledge of epigenetic pattern in periodontal diseases may add not only to the knowledge of susceptibility of the disease but may also be a diagnostic tool to identify patients at risk to develop the severe form of periodontitis. In addition, recent research on gene therapy and tissue engineering indicates a role for epigenetics to improve regeneration of periodontal tissues.

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