REVIEW ARTICLE

Role of Epigenetic Mechanisms in Oral Health: A Review

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Abstract

Aim: To review the literature on the oral health status of the population, evidence-based understanding of epigenetic mechanisms, and its significant role in diseases occurring in the oral cavity. **Background:** Epigenetics is the study of heritable changes in gene expression that does not involve changes to the underlying DNA sequence. In other words, it is a change in phenotype without a change in genotype, which in turn affects the way cells read genes. Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state. The field of epigenetics is quickly growing, and it is believed that both the environment and lifestyle can interact with the genome to influence epigenetic change. These changes may be reflected at various stages throughout a person's life and even in later generations. **Conclusion:** Epigenetic codes help us understand the biological phenotype that arises from the interaction of the human genome with the environment in health and in disease. Epigenetics is a major turn away from molecular biology. Current epigenetics not only offers new insights into gene regulation and heredity, but also it challenges the way we think about evolution, genetics, and development. Most interestingly, it suggests testable mechanisms whereby environmental factors (ranging from stress to infection) can influence genetic expression.

Key words: Oral health, epigenetics, epigenetic mechanism, DNA methylation, histone modification, Non coding RNA

INTRODUCTION

Despite enormous improvements in the oral health standards across several countries, the burden of oral diseases worldwide still persist.[1] Problems such as dental caries, periodontal disease, tooth loss, oral mucosal lesions and oropharyngeal cancers, human immunodeficiency virus/acquired immunodeficiency syndrome-related oral disease, and orodental trauma are major public health problems globally. The experiences associated with these diseases impact people's daily lives and well-being. The burden is particularly towering for the disadvantaged and poor population groups.[1]

Epigenetic mechanisms can regulate gene expression and affect the progression of certain diseases mentioned above. Epigenetics in dental research is at the early stages. However, it deserves attention because it plays an important role in gene expression during tooth development and may affect oral diseases.[2] Understanding epigenetic alterations are important for developing new treatment methods. At least three systems including DNA methylation, histone modification, and non-coding RNA (ncRNA)-associated gene silencing are currently considered to initiate and sustain epigenetic change.[3]

ORAL HEALTH STATUS

Global trends

Oral health is one of the essential parts of health-care system agenda in developed countries.[4,5] The WHO emphasized that despite great improvements in the oral health of populations in several countries, problems still persist.[6] Dental caries is on the rise largely due to the increasing consumption

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Received: 04-08-2016
Revised: 17-09-2016
Accepted: 20-10-2016

of sugars and inadequate exposure to fluorides affecting 60-90% of school-aged children and nearly 100% of the adult population. In 2004, WHO measured the severity of dental caries by the decayed, missing, and filled teeth index (DMFT).[7,8] Dental caries experience in children is relatively high in the Americas (DMFT = 3.0) and in the European Region (DMFT = 2.6), whereas the index is lower in most African countries (DMFT = 1.7), Latin America shows high DMFT values (i.e., 14 Worldwide).[6-8]

Tooth loss in adult life may also be attributable to poor periodontal health. Severe periodontitis, which may result in tooth loss, is found in 5-20% of most adult populations worldwide. The manifestation of periodontal disease is highly prevalent among adults in all regions.[7] Nearly all children and adolescents worldwide have signs of gingivitis. Aggressive periodontitis invades about 2% of youth.[9]

The Indian scenario

In India, negligence of oral health is apparent from the increased prevalence of oral diseases.[10] This impacts quality of life, especially among poor, vulnerable, and marginalized groups. This is promoted due to the prevalence of risk factors and inadequate access and affordability of preventive and curative oral health services, lack of definitive planning, and research which are the barriers to oral health encouragement.[10] Oral manifestations of smoking are common.[11] Excess amounts of fluoride in ground water are seen in nearly 17 Indian states accounting for endemic fluorosis. The distribution of dental caries among all age groups was 50%. The existence of malocclusion in India is estimated to be 30% in school-age children.[12] Around 66% of primary school and 59% of secondary school children suffer from at least one chronic disease.[13] About 50% of schoolchildren are estimated to have dental caries while it is believed that more than 90% of adults suffer with periodontal diseases.[13] Studies across the country exposed, 17% were edentulous, 78.3% had missing teeth, 1.5% were using dentures, and only 3.2% had intact teeth.[14] The WHO reported 19% edentulism among 65-74 age group.[5] In 35-44 years and 65-74 years, 100% prevalence for gingival bleeding was reported at Orissa and Rajasthan.[10] In the DCI survey report, oral precancerous conditions and oral cancer ranged between 3% and 10%. It was reported to be 7% prevalent in Orissa and only 0.3% in Delhi.[10,15]

Epigenetics

Conrad Waddington was the first to coin the term. According to him, epigenetics is defined as "the branch of biology that studies the causal interaction between genes and their product, which bring the phenotype into being."[16] Holliday described epigenetics as "the study of the mechanisms of temporal and spatial control of gene activity during the development of a complex organism."[18] According to Russo *et al*., epigenetics was defined as "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in the DNA sequence." The "epi-" in epigenetics translated from Greek means "on the top of" or "in addition to" genetics.[19] A consensual definition of epigenetics is described as follows "stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence."[20] These alterations manifest itself through three popular mechanisms known as DNA methylation, histone modification, and gene regulation by ncRNAs.[16,17,21-23] It must also be noted that epigenetic modifications are reversible and transient. Environmental stresses act as epigenetic modifiers.[24] Eventually, epigenetic alterations modulate gene expression and affect various gene functions.

EPIGENETIC MECHANISMS

Epigenetic mechanisms exert an additional layer of transcriptional control that regulates gene expression.[22] They are coupled and interact to modify chromatin structure and function. They occur throughout the lifetime of the organism, beginning in intrauterine environment, and accumulate in tissues and cells over time to modify gene expression patterns and cellular phenotypes.[22]

DNA methylation

Interaction between inherited genome and the dynamism imposed by the environment, promotes specific DNA methylation patterns,[25] referred to as metastable condition that represents an epigenetic modification, resulting in a new cellular or tissue homeostatic "set-point" with a new range of gene expression patterns. It is the most characterized type of chromatin modification involving the covalent transfer of a methyl group from S-adenosyl methionine (SAM) to cytosines present in cytosine-phosphate-guanine (CpG) dinucleotides of the DNA chain.[2] In normal cells, DNA methylation occurs predominantly in repetitive genomic regions including satellite DNA and parasitic elements (LINES and SINES, and endogenous retroviruses,[26] offering a mechanism by which the environment can stably change gene expression.

Significance

It constitutes missing link among genetics, disease, and the environment. Abnormal methylation, such as hypomethylation, leads to changes in DNA that are associated with chromosome instability and human cancers.[27] It is critical for proper regulation of the genome. It is recognized as an ancient host defense system, intended to protect against exogenous parasitic nucleic acid sequence elements, or deleterious endogenous sequences, which evolutionarily is integrated and retained vestigially within our genome.[27,28] Changes in methylation status can also regulate differentiation, cell growth, and cell death.[29,30]

DNA methylation in oral health

It can be altered by a persistent inflammation[31] and is also observed in chronic periodontitis.[32] Methylation patterns may be different between healthy and inflamed dental pulp.[33]

Histone modification

Post-translational modification of core histones, by either condensing or relaxing chromatin, regulates gene transcription making it a crucial epigenetic mechanism. The basic unit of chromatin, the nucleosome, consists of a DNA segment and eight core histones, making it a rigid structure. The modification of histones takes place mostly at the N-terminal tails of the protein.[34] Acetylation of the core histones relaxes the structure of chromatin.[21] In opposition, histone deacetylases (HDACs) cause the chromatin to condense and repress gene transcription.[21]

Significance

It is a powerful epigenetic mechanism since it can regulate gene expression by altering chromatin states through either acetylation or methylation.

Histone modification in oral health

It can induce differentiation and mineralization of dental pulp stem cells (DPSCs). It is also believed to promote pulp repair and regeneration playing a vital role in restorative dentistry.[35,36]

ncRNA

ncRNAs such as transfer RNAs, ribosomal RNAs, microRNAs (miRNAs), and short-interfering RNAs (siRNAs) functionally resemble RNA molecules although they do not code for protein. miRNAs and siRNAs regulate gene expression without altering the DNA sequence.

Significance

Guo *et al*., believe that miRNAs are involved in multiple vital processes throughout the development or differentiation of a disease.[21,23]

ncRNA in oral health

They are involved in specific syndromes and oral diseases such as oral cancer and oral immunology. Studies demonstrate that miRNAs aid in odontoblast differentiation.[37,38]

Environmental stressors as epigenetic modifiers

It has been reported that there are epigenetic changes during fetal development, progression of cancer states, or in chronic diseases such as autoimmune diseases, diabetes mellitus, cardiovascular diseases, and mental diseases in adults.[40] Microbial and viral exposures can change epigenetic patterns. Intrauterine nutrition can determine epigenetic programming of the fetus. Although many epigenetic marks are potentially reversible, many epigenetic changes appear to persist throughout the cell lineage and life of the organism. This can elucidate the Barker hypothesis,[41] which claims that fetal programming lingers throughout adulthood, contributing to the risk of cardiovascular disease and type 2 diabetes.

CpG methylation can be influenced by external stressors, environmental toxins, and aging, potentially increasing or decreasing the level of transcription.[42,43] Other environmental stimuli that potentially function as epigenetic modifiers is the exposure to metals and aromatic hydrocarbons (e.g., benzopyrene) found in occupational chemicals, fossil fuel emissions, polluted drinking water, cigarette smoke, and infection.[44] Smoking instigates changes in DNA methylation showing association of the offspring's DNA methylation with paternal DNA methylation was strongest if both had never smoked ($P = 0.02$).[45] In addition, smoking also causes hypomethylation and hypermethylation changes in DNA.[24] Smoking is linked to oncogenesis by inducing specific epigenetic modifications.[46] During pregnancy, maternal folate deficiency causes inadequate levels of SAM.[47] Consequentially, maternal folate deficiency can lead to DNA hypomethylation, which can cause excessive expression of certain genes and genetic instability in the fetus.[48] Brook proposed that dental anomalies are results of genetic–epigenetic interactions.[49] Several nutritional factors such as folate, Vitamin B12, and Vitamin A may lead to changes in epigenetic modification. Therefore, some exogenous factors such as diet, smoking, environment, bacteria, inflammation, and age may affect oral health by causing epigenetic changes. These deficiencies culminate in growth defects and birth anomalies.[50] Sensitivity to diet or to environmental stress varies due to former genetic variants challenging methyl metabolism and making individuals susceptible to epigenetic changes.[51]

EPIGENETICS IN DENTISTRY

Epigenetics is particularly evident in the context of dental health.[24,39,51-56]

Epigenetics in development of oral cavity

Epigenetic factors present at each developmental stage can affect the developmental processes. Epigenetic mechanisms also play a crucial role in tooth development. Histone demethylase may regulate the dental stem cell differentiation.[39] In addition, histone acetyltransferase and ncRNAs may influence odontogenic differentiation.[37]

Fan *et al*. found that the oculofaciocardiodental syndrome, which is characterized by canine teeth with extremely long

roots, is associated with a BCL-6 corepressor mutation.[51] This mutation leads to the upregulation of AP-2a in mesenchymal stem cells and promotes osteodentinogenesis.

Dentinogenesis

Expression of miRNAs is elevated during the last stages of osteoblast differentiation, demonstrating the importance of miRNA regulation for attenuating continued bone formation at the final osteocyte stage of differentiation.[52,53] Dlx3 collaborates with transcription factors unique to mineralized tissue to regulate craniofacial and postnatal skeletal development.[54-56] Dlx3 stimulates dentinogenesis. The posttranscriptional regulation of Dlx3 by miR-665 controlled by BMP2 and RUNX2, implies an combined network of signaling and transcription factors that coordinate the stage-specific events of odontoblast differentiation.[57,58] KAT6A promotes RUNX2 acetylation to increase the transcription of genes involved in dentinogenesis. The multifunctional role of miR-665 highlights its function in controlling differentiation and tissue development.[59-61] miRNAs link genetic and epigenetic events that are requisite for maintaining a normal tissue environment. The involvement of miR-665 at multiple levels of odontoblast differentiation suggests a therapeutic role for dental disorders.

EFFECT OF ALCOHOL ON ADULT STEM CELLS

Lysine-specific demethylase 6B (KDM6B) was significantly dysregulated in DPSCs upon EtOH exposure. EtOH treatment during odontogenic/osteogenic differentiation of DPSCs suppressed the induction of KDM6B with alterations in the expression of differentiation markers. The study has demonstrated that EtOH-induced inhibition of KDM6B plays a role in the dysregulation of odontogenic/osteogenic differentiation in the DPSC model. This suggests a potential molecular mechanism for cellular insults of heavy alcohol consumption that can lead to decreased mineral deposition potentially associated with abnormalities in dental development and also osteopenia/osteoporosis, hallmark features of fetal alcohol spectrum disorders.[62]

EPIGENETIC GENE REGULATION BY HISTONE DEMETHYLASES:

KDM2A has been found to promote ameloblast and odontoblast differentiation and suppress adipogenic and chondrogenic differentiation of stem cells from the apical papilla, indicating its importance in tooth development.[63,64] Zheng *et al*. demonstrated that KDM5B and KDM6B are upregulated during tooth development.[65] KDM6B has been shown to transcriptionally activate bone morphogenetic protein 2 expression and promote odontogenic differentiation of dMSCs and mineralized tissue formation.[66] KDMs play an important role in alveolar bone formation and craniofacial development. Yang *et al*. showed that KDM6A transcriptionally activates runt-related transcription factor 2 (RUNX2) and Osterix important transcription factors for osteoblast differentiation, thereby promoting mineralized tissue formation. Xuan *et al*.[67] showed indirect evidence of KDM6B involvement in periodontal inflammation through regulation of M2 macrophage polarization although the detailed mechanisms by which KDM6B regulates periodontal tissue responses to infection remains unknown.[68]

MANIFESTATION OF EPIGENETIC MECHANISMS IN ORAL DISEASES

Periodontics

It is a complex infection characterized by inflammation and destruction of the tooth-supporting tissue. Gene expression was altered by epigenetic modifications in periodontitis.[71] Gomez *et al*.[31] reported that the methylation pattern caused by changes in cytokine gene expression could lead to inflammatory diseases. Inflammatory cytokines such as interleukin 1 (IL1), IL4, IL6, and IL10 are found to be overexpressed in the inflamed periodontal system.[72] Stenvinkel *et al*. suggested that a persistent inflammation leads to DNA methylation, silencing the suppressors of cytokine signaling, and inducing the active expression of cytokine signaling.[73] Cytokines such as IL6 and interferon gamma (IFNγ) were found to be overexpressed in inflamed tissues of chronic periodontitis patients.[70,74] Zhang *et al*. reported that hypomethylation of the IFNγ promoter may lead to increased IFNγ transcription in chronic periodontitis, thus resulting in IFNγ overexpression.[70] In contrast, genes such as tumor necrosis factor alpha (TNFa) and cyclooxygenase-2 were hypermethylated at the CpG site which represses expression.[71] Zhang *et al*. reported that the change in the methylation patterns may be a crucial factor in regulating TNFa transcription in periodontitis.[70] In addition, it was found that chronic inflammation is related to altered DNA methylation levels.

Inflammation

Inflammation is a biological response to noxious stimuli such as pathogens or irritants. It brings about epigenetic changes including DNA methylation and histone modification.[21,22,24,69] It is reported that inflammatory signals promote the activity of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-kB), thereby potentially modifying histone methylation patterns and promoting gene expression.[61] Periodontitis infected pulp and periodontal tissue may alter gene expression patterns of inflammatory cytokines.[70-73,33,75] Therefore, these epigenetic biomarkers are to be determined for prevention and treatment of dental diseases. The link

between inflammation and oral cancer is well established, and the connection between bacterial infection and inflammation is evident.[77] Thus, epigenetic influences may serve as a plausible potential mechanism that connects all three pathways and should be further explored, especially as it relates to mucosal cancers, which emerge in the presence of high microbial burdens.

Oral squamous cell carcinoma (OSCC)

The best-studied epigenetic alteration in cancer is DNA methylation.[22] In most tumors, hypomethylation occurs, which increases transcriptional activity. It is the earliest epigenetic modification signifying changes from normal to pre-malignant cells.[22] It is associated with 25-52% of primary oral squamous cell carcinomas.[78-80] Choi and Myers[81] emphasized the role of oncogenes such as Ras oncogene, Cyclin D1, AP-1 complex, and tumor suppressor genes such as p53, p16, and p21 in the molecular pathogenesis of OSCC. Aberrant hypermethylation patterns in the promoter region of p16 and E-cadherin influences cell division and cell–cell adhesion, respectively. E-cadherin plays a role in cell–cell adhesion, and, when underexpressed, may affect tumor invasion by leading to a greater probability of tumor invasion or metastasis. E-cadherin was found silenced by hypermethylation in other studies of oral cancer.[80,82,83] During tumorigenesis, methylation is usually decreased genome-wide, with selective hypermethylation of CpG sites within promoters of tumor-suppressor genes, leading to their silencing and subsequent tumor progression.[84] This suggests that oncogenesis may also occur through epigenetic dysregulation. Feinberg[85] claimed that epigenetic modifications may play a role in cancer predisposition and that such changes should be considered as targets for preventive oncology.

OSTEOGENIC DIFFERENTIATION OF HUMAN PERIODONTAL LIGAMENT (HPDL) CELLS

One of the characteristics of PDL cells is their plasticity. One possible mechanism might be related to epigenetics since HDACs have been shown to play a role in osteoblast differentiation. In the presence of the HDAC inhibitor, osteogenic differentiation was induced; osteoblast-related gene expression was increased significantly. During osteogenic differentiation, HDAC 3 expression gradually decreased. This was apparent in the absence and presence of the inhibitor. The level of acetylated histone H3 was increased during osteogenic differentiation. Inhibition of HDAC activity induced hyperacetylation of histone H3, therefore, demonstrating histone H3 as a candidate target molecule for HDAC inhibition. In conclusion, hPDL cells express a distinguished series of HDACs and these enzymes appear to be involved in osteogenic differentiation. This finding suggests a potential application of trichostatin A for bone regeneration therapy by hPDL cells.[86]

EPIGENETICS IN IMMUNE RESPONSE

Periodontitis is a chronic inflammatory disease triggered by the host immune response.[86] Epigenetic modifications also affect the immune response. In gingival biopsies taken from patients with AgP, CpG methylation of CCL25 (1.73% vs. 2.59%, $P = 0.015$) and IL17C (6.89% vs. 19.27%, $P = 0.002$) was significantly reduced as compared with periodontally healthy tissues. CCL25 plays an important role in T-cell development, whereas IL17C regulates innate epithelial immune responses. The decrease in CpG methylation is presumably accompanied by an increase in gene expression. This could lead to a greater availability of CCL25 and IL 17C and support periodontal loss of attachment.

FUTURE RESEARCH

The role of epigenetics and its oral manifestations is fertile ground for future research. It holds exciting new discoveries yet to be revealed. It will enable us to widen our understanding of how epigenetic patterns affect the expression of oral conditions such as oral cancer or advanced periodontitis. We need to understand how the oral microbiome and local biofilm may create an epigenetic "footprint" in the adjacent mucosa and periodontal tissues and potentially modify the local inflammatory response and oncogenic potential. Epigenetic remodeling of cells to a pluripotent state proposes that epigenetic reprogramming might prove to be a mechanism to create new wound healing or tissue regenerative potential, and agents which modify epigenetic patterns. This is a thrust area for new drug development strategies. These are questions which await further studies to explore the role of epigenetics in oral biology. Future researches are required to corroborate initial studies through which better understanding of the effect of epigenetics on dental health, and development of effective therapies may be established.

CONCLUSION

Oral diseases are major public health problems in all regions of the world.[4] Their impact on individuals and communities as a result of the pain and suffering, impairment of function, and reduced quality of life they cause, is considerable. Epigenetics significantly affects oral health.[1] Epigenetics plays an important role in gene regulation.[2] Although studies focused on epigenetics in dentistry are still in the early stages, there is increasingly more evidence for the association between epigenetic changes and periodontal diseases,[31] as well as inflamed dental pulp cells. Popular epigenetic mechanisms include DNA methylation,[47] histone

modification,[21] and ncRNAs.[23,38] Epigenetic changes can contribute to the progression of certain diseases such as cancer[44] and these mechanisms are also susceptible to modification by factors ranging from infection to stresses such as diet, smoking,[46] stimuli, and inflammation.[50]

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Source of Support: Nil. **Conflict of Interest:** None declared.