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Research Article

MICRO-RNA - A FORMIDABLE ENTRANT IN PATHOLOGY OF PERIODONTAL DISEASE

Krutika Yagnik and Jaideep Mahendra

Department of Periodontology Meenakshi Ammal Dental College, No. 1, Alapakkam main road, Maduravoyal, Chennai - 6000095

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ABSTRACT

Periodontal disease is influenced by various etiological factors which may be microbiological, environmental, host modulatory or genetic in nature. The fact that genome plays an important role in periodontitis is seen in form of varied host response by different individuals even in presence of similar microbiota and environmental influence. Micro-RNA is one of the recently discovered transcriptomes that influences various developmental processes ranging from cell development, differentiation, proliferation as well as death. It mainly acts on messenger RNA and influences the protein translation process. While there have been conclusive evidences of role of micro-RNA in disease processes such as cancer, cardiovascular disease, diabetes mellitus, Alzheimer's etc, study of its role in periodontitis is just beginning to evolve. This review highlights the role of micro-RNA in a chronic inflammatory pathology which is Periodontitis, its effect on the immune mechanisms, bone regulation and its defining role as biomarkers of disease process.

INTRODUCTION

Genomic studies in the recent years have offered novel insights into possible genetic influences and regulators of periodontitis. One of the promising candidate under scrutiny of researchers has been Micro-RNA. Micro-RNAs (miRNA) are a family of small (22 base-long) non-coding RNA molecules which play an important role in regulation of gene expression in both animals as well as plants. They act to repress their target genes and affect protein translation. Micro-RNA induced dysregulation may lead to development of many chronic inflammatory diseases including periodontitis. The expression patterns and distribution of micro-RNAs shows a high degree of specificity in periodontal disease.

History of Micro-RNA

The insight into presence of micro-RNA was first given by Victor Ambros, Rosalind Lee and Rhonda Feinbaum who discovered *lin-4*. This was a gene known to control the timing of larval development in bacteria *C. elegans*.[1] It was found that this gene did not code for protein but instead produced a pair of small RNA's - one that was 22 nucleotide(nt) in length. This short *lin-4* RNA is now recognised as founding member of class of tiny regulatory RNA's called Micro-RNAs. Discovery of *let-7*, another gene in *C.elegans* heterochronic pathway which encoded a second 22 nt regulatory RNA further brought focus into these tiny non-coding RNA's. Micro-RNAs were not recognized as a distinct class of biological regulators until the early 2000s. The study on plants by Palatnik *et al*.[2] provided one of the most compelling cases that the newly discovered micro-RNAs have an important role in controlling development. Further scrutiny into these transcriptomes have revealed numerous additional miRNA genes in mammals, fish, worms and flies. A special registry called miRBase has also been set up to catalogue the miRNAs and facilitate mapping of newly identified genes.[3] Most of these miRNAs genes are derived from independent transcription units while a sizable minority are in the introns of precursors of messenger RNAs (mRNA). This therefore explains the close relationship between mRNA and miRNA.

Biogenesis of Micro-RNA

Micro-RNAs are processed from genes that are much longer than the final miRNA transcriptome.These primary transcripts from which miRNA are transcribed are called Pri-miRNA.[4] Micro-RNAs are transcribed by RNA polymerase II and comprise of a 5' cap and poly-A tail. The pri-miRNAs are acted upon in the nucleus by the microprocessor complex, consisting of the RNase III enzyme Drosha, and the double-stranded-RNA-binding protein, Pasha/DGCR85.[5] This results in

Corresponding author:* **Krutika Yagnik
Department of Periodontology Meenakshi Ammal Dental College, No. 1, Alapakkam main road, Maduravoyal, Chennai – 6000095

formation of Pre-miRNAs which resemble stem loops intertwined imperfectly and these are approximately 70 nucleotide in length. The pre-miRNAs are then transported into the cytoplasm. Once in the cytoplasm, the pre-miRNAs are further cleaved by the RNAse III enzyme Dicer generating the Micro-RNA, a double-stranded RNA approximately 22 nucleotides in length. RNA-induced silencing complex (RISC) is also formed via Dicer. This is responsible for the gene silencing observed due to miRNA expression and RNA interference.[6] (Figure 1)

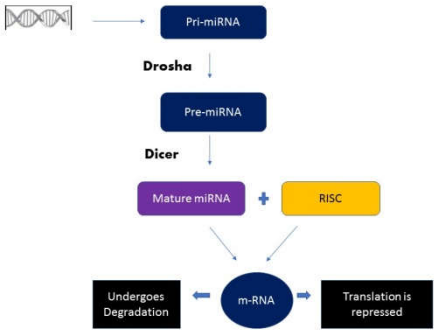

Figure 1 Biogenesis of Micro-RNA

Mechanism of action of Micro-RNA's

Micro-RNA's control gene expression post-transcriptionally by regulating mRNA translation or their stability in the cytoplasm. The micro-RNAs function to either repress or degrade mRNA molecules. This occurs through base-pairing with complementary sequences within mRNA molecules. As a result, these mRNA molecules are silenced, by either cleavage of the mRNA strand into two pieces, by destabilization of the mRNA through shortening of its poly(A) tail, or by less efficient translation of the mRNA into proteins by ribosomes. Thus, by these mechanisms, each miRNA, not only directly represses a number of target genes, but also indirectly affects other associated genes to regulate cell functions.[7] Just as micro-RNA is involved in the normal functioning of eukaryotic cells, similarly dysregulation of micro-RNA has been associated with disease processes. Involvement of micro-RNA in diseases such as cancer, myocardial infarction, diabetes mellitus, nephrotic syndrome has been well documented in the past.[8,9,10,11]

Micro-RNA and Dentistry

Micro-RNA is an integral component of various dental tissues. Studies have shown it plays an important role in embryogenesis, development of tooth bud as well as various dental tissues like dental pulp and periodontal ligament and alveolar bone.[12]Alteration in miRNA profiles also results in inflammatory conditions of dental pulp, periodontal ligament and alters the bone metabolism. There has been advent of new literature showing the role of micro-RNA in the field of orthodontics influencing tooth movement by its regulatory action on bone.[13] Similarly micro-RNAs have also been implicated in development of pulpal disease by their specific and intricate role in essential aspects of pulpal pathology, i.e. inflammation and immunity.[14] Micro-RNAs also play an important role in field of oral medicine in diagnosis of various oral pathologies especially in early detection of oral carcinomas.[15]

MICRO-RNA and Periodontal disease

Proteins comprise one of the main components of various tissues of periodontium. These are involved in various functions such as cell matrix adhesion and signalling the diffusion of nutrients, waste products, imparting compressive and tensile strength to the tissues, allowing for growth and development of tissues as well acting as soluble signalling molecules during inflammatory cascade. Micro-RNA's are present in all of the periodontal tissues since the time of embryogenesis. These basically function to either alter or degrade the mRNA translation of various proteins in periodontium. Thus, dysregulation in protein functions or structure may lead to various disorders in periodontium. Micro-RNAs play various roles in complex biological cascades including the inflammatory responses. The shift in miRNA profiles in the gingiva, indicates the miRNA-related host immune response to oral bacterial infection.Various studies have investigated the profile of miRNAs in inflamed gingiva in comparison with healthy tissue (Table 1) These studies show that micro-RNAs have potential targets in gingival tissue and play an important role in gingival inflammation.

Table 1 Various studies that have elicited the role of Micro-RNA in periodontal disease

Study	Population/Sample	Results
Lee *et al.*[16]	Normal healthy gingiva and diseased gingival tis-sues were obtained from patients	miR-181b, miR-19b, miR-23a, miR-30a, miR-let7a, and miR-301a were upregulated in diseased gingiva compared to healthy gingival
Xie *et al.*[17]	Gingival tissues were obtained from 10 healthy and 10 periodontitis affected patients	Ninety-one miRNAs were found to be upregulated and thirty-four downregulated over two-fold in inflamed gingival tissue compared with the healthy tissue. Twelve selected inflammatory-related miRNAs, miR-126, miR-20a, miR-142-3p, miR-19a, let-7f, miR-203, miR-17, miR-223, miR-146b, 146a, miR-155, and miR-205 showed significant upregulation.
Na *et al.*[18]	Gingival tissues from patients with periodontitis and those with healthy gingiva were collected	miRNA-128, miRNA-34a, and miRNA-381 were upregulated in periodontitis patients whereas miRNA-15b, miRNA-211, miRNA-372, and miRNA-656 were downregulated.
Ogata *et al.*[19]	Inflamed and non-inflamed gingival tissues were obtained from patients	miR-150, miR-223, and miR-200b were overexpressed in inflamed tissues whereas miR-379, miR-199a-5p, and miR-214 were underexpressed.
Wasmer *et al.*[20]	158 diseased and 40 healthy gingiva were assessed and expression of 1205 miRNAs were assessed in the tissues.	159 miRNAs were significantly differentially expressed between healthy and diseased gingiva. Four miRNAs: miR-451, miR-223, miR-486-5p, miR-3917 were significantly overexpressed, and 7 miRNAs: miR-1246, miR-1260, miR-141, miR-1260b, miR-203, miR-210, and miR-205 were under expressed by > 2-fold in diseased vs. healthy gingiva.

Micro-RNA and its role in Periodontal inflammation

Inflammation is a complex protective process that requires interactions between different types of immune cells to remove or neutralize harmful stimuli. Neutrophils, dendritic cells, and macrophages express Toll like receptors(TLR) participate in the transmission of a signal from the plasma membrane, through a multistep cascade to the responsive transcription

factors. Binding of the TLRs to their respective ligands initiates a wide spectrum of responses, from phagocytosis to production of a variety of cytokines, which in turn shape and enhance the inflammatory and adaptive immune responses. Typical transcription factors that activate inflammatory mediators are Nuclear factor kappa B (NF-κB), activator protein 1, signal transducer and activator of transcription (STAT), CCAAT enhancer binding protein (C/EBP), and Ets-like gene 1. Micro-RNA 146-a and Micro-RNA 155 play an important role in regulation of immune responses through toll like receptors. Micro-RNA 146a negatively regulates Toll-like receptor as well as cytokine signalling by down-regulating IL-1 receptor-associated kinase 1 and TNF receptor-associated factor 6 protein levels.[21]It also negatively regulates TLR2-induced inflammatory responses in keratinocytes and macrophages.[22] **Wang Z *et al*** showed that both miRNA 146-a and 155 effectuate the inflation of response by toll like receptors that is triggered by leukotriene B4,which is one of the most potent stimulants of mononuclear cells.[23] Micro-RNAs also play a major role in both humoral as well as cell mediated inflammatory responses. Micro-RNA 146-a shows upregulation in periodontitis by earmarking lymphocyte transcription factor Bob1.[24] Similarly, micro-RNA 155 shows upregulation in periodontitis by inducing the differentiation of Th1 cells and by inhibiting interferon-gamma signalling.[25] Apart from these two miRNAs, other miRNAs associated with adaptive immune responses include miRNA-210 and miRNA-650. While miR-650 is upregulated in periodontitis by influencing proliferating capacity of B cells through its targets on cyclin-dependent kinase 1 (CDK1) [26], miR-210 is found to be downregulated since it impairs FOXP3 protein which regulates T helper cells.[27] Micro-RNAs also regulate the pathways of dendritic cell signalling. miR-155 reduces the c-Fos expression which negatively affects its concomitant dendritic cells.[28] miR-148 and miR-152 also target proteins involved in calcium regulation and impairs dendritic cell signalling as well as its antigen presenting capacity.[29]

Micro-RNAs and Periodontal pocket

Periodontal pocket formation is a key clinical feature of the periodontal disease state. The periodontal pocket histologically is composed of various pathogens that invariably lead to an environment of low oxygen states. This promotes the growth of more virulent group of organisms and leads to progression of the disease. The hypoxic state reveals a specific group of micro-RNAs called as hypoxamirs which either further augment the cytokine production by the virulent organisms in the periodontal pocket or may limit the hypoxic effect. Micro-RNA 155 and 210 are key miRNAs present in hypoxic states. miR-155 triggers cell cycle arrest and attenuates cell proliferation through its targeting of MTOR pathway, which is a key signalling pathway involved in regulation of cell cycle.[30] miR-210 controls the host response to hypoxia, by targeting Hypoxia inducible factor-α (HIF-α) which limits the duration of the hypoxic reaction and the disease process.[31]

Micro-RNAs and Alveolar bone metabolism

Chronic periodontitis is not a continuous occurring disease process and is characterized by periods of aggravation as well as period of quiescence. Bone remodelling in chronic periodontitis is balanced by bone formation and resorption, modification in quantity of seed cells which lead to either the maintenance or worsening of the bone status. Micro-RNAs have been verified to play vital roles in the regulation of osteogenesis and osteoclastogenesis, and they interact with signalling molecules to control these processes. Micro-RNAs are vital for differentiation of the mesenchymal stem cells into their lineage of osteoblasts or osteoclasts.Absence of miRNA during developmental stages and adult stages leads to impaired osteoblast differentiation and later delayed mineralization.[32] MicroRNAs such as miR-23a, miR-133a, miR-135a, miR-137 inhibit osteoblast differentiation in premature osteoblasts by directly targeting Runt related transcription factor 2 (Runx2).[33] miR-34a acts as an inhibitor of osteoclast differentiation by causing repression of TGF-β genes.[34] Analysing the same **miRNA, Krzeszinski *et al***.[35] showed that miR-34a delivery into animal model increased the bone quantity and lowered the bone metastatic rate, thus confirming the osteogenic role of miR-34a. Several miRNAs have also been reported to be involved in the regulation of receptor activator of NF-KB ligand (RANKL) expression in various cell types. The level of RANKL expression appears to be associated with osteoclastogenesis. It is seen that miR-106b inhibits osteoclastogenesis and osteolysis by directly targeting RANKL.[36]

Micro-RNA regulated bone remodelling, apart from targeting seed cells also targets the bone metabolic pathways. miR-29a acts on Wnt signalling pathway in human osteoblasts and directly targets three negative regulators of Wnt signalling, Dkk1, Kremen2, and secreted frizzled related protein 2 (sFRP2), leading to osteoblast differentiation.[37] MiR-31 targets Osterix, which causes impaired osteoclast formation and reduces bone resorption.[38] Likewise, it is observed that miR-200c targets Notch signalling pathway and inhibits the Notch-1 ligand Jagged1 and thereby represses odonto- and osteoclastogenesis.[39] This miRNA is found to be downregulated in periodontitis affected gingiva which may explain the excessive bone loss in periodontal inflammation.

Micro-RNA and Periodontal regeneration

Due to the conserved nature of micro-RNAs as well as their constant expression in the disease process, they also assume significance for use in tissue engineering for achieving more predictable outcomes for periodontal regeneration. **Annalisa P *et al*.**[40] used micro-RNA microarrays techniques to investigate differences in translational regulation in osteoblasts exposed to bio-oss and perioglass, two biomaterials used for periodontal regeneration. Micro-RNA expressions showed that perioglass caused activation of bone forming miRNAs while bio-oss activated cartilage inducing miRNAs. This study showed how micro-RNAs could be used to understand the molecular mechanisms of various biomaterials. In future, this could help us in selecting appropriate materials that employ favourable strategies for periodontal regeneration.

Micro-RNA, Periodontitis and Systemic disease

Periodontitis and systemic diseases often have a bidirectional relationship. Significant associations between periodontal disease and cardiovascular disease, diabetes mellitus, preterm low birth weight, and osteoporosis have been discovered, bridging the once-wide gap between medicine and dentistry. These associations are based on the fact that most of these diseases like periodontitis share a common nature of slowly

progressive inflammatory and host modulatory pathways. The study of micro-RNA as a common regulatory factor between these diseases has been the focus of recent research. **Hai-Tao Sun *et al***.[41] stated that micro-RNA plays a role in co-development of metabolic syndrome and periodontitis. miRNAs associated with periodontal inflammation in obesity are noted to have predicted target mRNAs that regulate glucose and lipid metabolism, as well as the inflammatory response, and miR 185, a micro-RNA that induces DNA apoptosis is found to be upregulated in obese individuals with periodontitis as compared to non-obese group with periodontitis.[42]

Micro-RNA profiling and its role as periodontal biomarker

MicroRNAs have shown high amount of stability in formalin-fixed, paraffin-embedded gingival tissues and circulating miRNA's also show good stability in serum or plasma samples. Thus, they prove to be excellent biomarkers of the existing gingival or periodontal condition as well as indicators of any risk for future disease. Microarray profiling, real-time PCR array, and next-generation sequencing (NGS) technologies are used in initial screening of circulating miRNAs and generated miRNA signatures from body fluids. Newer diagnostic approach is use of salivary samples. Use of salivary miRNAs as non-invasive diagnostic markers has been studied in context of oral cancer, precancer, oesophageal cancer, and parotid gland cancer so far. The miRNAs in saliva have certain advantages as compared to other salivary biomarkers like proteins, mRNAs, DNAs and bacterial products due to the distinctive function of miRNA as post-transcriptional regulator, its stable nature in saliva and the similarity between miRNA profiles of saliva and other body fluids.[43] Saliva sampling has also proven to be non-invasive, economical and easy-to-access alternative in contrast to traditional tissue and blood sampling.Micro-RNAs also form complexes with other proteins and these complexes can also be found encapsulated in cell-secreted exosomes, from where the miRNA sequences can be recovered. Sometimes these vesicle-bound miRNAs can enter other cells where they can exert biological functions, essentially acting as biological messengers.

Future trends

Micro-RNA as therapeutic agents in periodontal disease

Micro-RNAs have a strong potential to be used for periodontal therapeutics. As these trancriptomes have special characteristics of being conserved in each species with known sequences, these can be positively used for making drugs. Origin of an oligonucleotide called anti-miR based on antisense technology has provided much needed boost to this research. This specific oligonucleotide targets its known miRNA and thus influences its functioning. This has been in trial stages for use in cancer. Similarly using the same principles development of miRNA therapy that potentially targets either the miRNA of the involved micro-organisms in periodontal disease and inhibits its expression or overexpresses the miRNA that lead to healthy gingival states, miRNA can be employed in periodontal therapeutics.[44] Researchers are now focussed on developing a design for an active synthetic miRNA which could bind to its respective targeted miRNA with high affinity and specificity, provide ease of administration through local or parenteral injection routes which would allow its sufficient uptake in the tissue. This could prove to be a game changer in treating various disease processes in the future.

CONCLUSION

In periodontal disease, the expression of miRNAs is highly specific. The recent development of new technologies to identify miRNAs has opened new avenues for diagnosis, prognosis and therapeutic applications.

While presently the understanding of miRNAs in biology of periodontal disease is still in its infancy, in future further research into these regulatory transcriptomes would definitely yield new insights into our understanding of immunophysiology and immunopathology of the disease process and lead to development of newer therapeutic strategies to identify the disease at an early stage and thus, either prevent or treat periodontal disease.

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