

Possible mechanisms of action of microRNAs in periodontal disease

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ABSTRACT

Objective: the purpose of this study was to review the role of possible mechanisms of action of microRNAs in periodontal disease. **Material and Methods:** the study was based on PubMed scientific papers. **Results:** the recent discovery of microRNAs (miRNAs) has revolutionized the way that gene regulation is analyzed. Studies demonstrate that they can act on the innate response, in several stages, among them the signaling of Toll-like receptors. The miRNAs are also related to the regulation of central elements of the adaptive immune response, such as antigen presentation. However, much still needs to be studied to identify miRNAs activity in gene regulation. Periodontal disease is an oral disease caused by bacterial pathogens, with an aggressive immune and inflammatory response, which affects the tissues around the teeth, which may lead to their loss. **Conclusion:** it was concluded that the recent findings of the role of microRNAs in the inflammatory response, including both innate and adaptive immunity, that occurs in periodontal disease.

Keywords: Periodontal disease; Inflammation; miRNA; Immune response; Periodontitis.

Introduction

Recently discovered, microRNAs (miRNAs) caused a paradigm shift in the understanding of genome expression and function, which was the first of these described in *Caenorhabditis elegans* nematodes in 1993. They are small, non-coding RNAs that are conserved throughout evolution, capable of regulating expression of genes at the post-transcriptional level through the degradation or repression of translation of messenger RNA (mRNA) target molecules into their 3' region.^{1,2}

Several functions have already been attributed to miRNAs, both in physiological processes, such as immune response, cell proliferation, and differentiation, apoptosis and transcriptional response and regulation, as well as in pathological processes such as autoimmune, cardiovascular and oral diseases and cancer predisposition and progression.^{3,4,5}

miRNAs are expressed in different cells of the innate immune system, such as monocytes, macrophages, dendritic cells, granulocytes and Natural Killer (NK) cells, which act as the first line of defense against various types of pathogens. There is ample evidence to suggest that miRNAs play key roles in the development and function of innate immune cells. Also, miRNAs also exert function on the adaptive immune system, several studies have demonstrated the purpose of these types of RNA in the development and dif-

ferentiation of T and B cells.⁵⁻⁸

Periodontal disease is caused by an immune and inflammatory response of the host to a bacterial infection that begins in the tooth, and this response relates to both innate and adaptive immunity that works together to limit infection and reestablish periodontal homeostasis.⁹

Although several studies have reported the role of miRNAs in the regulation of the immune response in viral and parasitic infections^{4,10,11,12} regulation of miRNA during bacterial infections, as in periodontal disease, needs to be further elucidated. Therefore, this study aims to review the recent findings of the role of microRNAs in the inflammatory response, including both innate and adaptive immunity, that occurs in periodontal disease.

Material and Methods

A non-systematic literature review was carried out to organize and discuss the central studies that investigate the relationship between microRNAs, inflammation, and periodontal disease. The consulted database was PubMed (www.ncbi.nlm.nih.gov/pubmed), were selected original articles that studied humans or animals, written in the English language and the following keywords were used to research: microRNA, immune response, bacterial infection, periodontal disease, inflammation, and ncRNAs.

Results

MicroRNA (miRNA)

miRNAs are defined as small endogenous molecules of ribonucleic acid (RNA) single-stranded, non-coding, with approximately 22 nucleotides, which act as regulators of gene expression at the post-transcriptional level through the cleavage of a target messenger RNA (mRNA) or translation repression.¹³

The microRNAs can control 30% of the expression of genes that encode proteins since a single one can regulate more than one target of mRNA. Thus, miRNAs are a mechanism of great importance in the process of gene regulation, since they are some of the most abundant classes of regulatory genes in humans.^{14,15}

The biogenesis of the miRNAs begins with their transcription in the nucleus. At this stage, they are called pri-miRNA, which can be many kilobases long. The RNA nucleotides within the pri-miRNA transcript interact with each other to form complementary pairs, causing the RNA to form secondary structures. The essential secondary structure is known as the stem-loop, also known as the hairpin, which is generated when two complementary stretches of RNA base-pair to form the “stem,” with an intervening set of RNA nucleotides that do not interact, which become the circular “loop”.¹⁶

The intramolecular hairpin structures are recognized by a protein complex that includes the Drosha enzyme, whose function is to excise the hairpin structures from the primary transcript. The excised hairpins are then called precursor microRNAs (pre-miRNAs) which are recognized by a protein, exportin-5, that brings them from the nucleus into the cytoplasm.¹⁶

In the cytoplasm, the pre-miRNAs are processed by Dicer, which removes the loop in the stem-loop structure, resulting in a double-stranded RNA duplex [17]. This RNA duplex is incorporated into the RNA-induced silencing complex (RISC), inside which the two RNA strands are separated. One of these strands remains associated with RISC and constitutes the mature miRNA, whereas the complementary strand undergoes to degradation.¹⁶⁻²¹

The miRNA and RISC complex (miRISC) regulate gene expression by acting on the mRNA degradation or repressing its translation. Depending on the degree of complementarity between the bases of miRNA and mRNA two regulatory pathways may be used: the small interfering RNA (siRNA) pathway, when the complementarity between mRNA and miRNA is almost perfect, and the path of miRNA, when this complementarity does not occur, this pathway is where most human mRNAs are processed.^{15,16,19}

miRNA and Immune Response

During the last decade, microRNAs (miRNAs) have

emerged as critical regulators of the immune response based on their ability to interfere with the post-transcriptional expression of multiple target genes.⁹

The immune response involves the induction of hundreds of genes, a process that must be well regulated to eliminate the pathogen and, at the same time, avoid the consequences of unregulated gene expression, that can lead to uncontrolled inflammation, tissue damage and even cancer.²⁰

Immune cells express a variety of pathogen recognition receptors, such as the Toll-Like receptors (TLR), which are discovered at the end of the 20th century and comprise a highly conserved transmembrane proteins that play an important role in detection and recognition of microbial pathogens, as well as the generation of signals to the production of pro-inflammatory proteins and cytokines.^{5,21}

Activation of innate immunity from the association of TLR with pathogen-associated molecular patterns (PAMP) of infectious agents is indispensable in the development of acquired immunity against specific antigens. Studies with several TLRs demonstrate that they activate the NF- κ B pathway, which regulates the expression of cytokines, through various molecules. On the other hand, activation of the NF- κ B pathway leads to initiation of adaptive immune response by the production of inflammatory cytokines such as IL-1, IL-8, IL-12, tumor necrosis factor- α (TNF- α) and the induction of co-stimulation molecules, such as CD80, CD86, and CD40.²²

Although inflammation plays an important role in fight infection, its deregulation often occurs and can cause a variety of pathologies. Recent evidence points a fundamental role of miRNAs in the coordination of certain characteristics of the inflammatory process, such as regulation of central elements of the adaptive and innate immune response, since studies show that they are able to act in the antigens presentation (miR-115 and miR-181) and also regulate TLR and cytokine signaling (miR-146).⁶

miR-146a regulates innate immune, inflammatory response, and antiviral pathways negatively and is aberrantly expressed in various diseases.^{5,23}

Taganov *et al.*²⁴ demonstrated that miR-146 expression in human monocytes was observed in response to a variety of microbial components and proinflammatory cytokines. The rapid induction of miR-146 in response to LPS, as shown by Real-Time Polymerase Chain Reaction (qPCR), suggested that miR-146 may be a primary LPS response gene. Based on this analysis, was proposed a role for miR-146 in the control of TLR and cytokine signaling involving TNF and the down-regulation of IL-1 associated with the kinase-1 receptor. The results were sufficient to conclude that miR-146 is an immediate early response gene, induced by various microbial components and proinflammatory mediators.²⁴

However, O'Connell *et al.*²⁵ stated that miR-146a is a neg-

ative regulator of the immune response. This occurs by inhibiting the expression of the mRNAs that encoding TRAF6 and IRAK1, two proteins that are involved in the transduction of TLR signaling leading to the activation of NF- κ B. During and after cell activation of the innate immune response, miR-146a decreases the production of inflammatory mediators such as IL-6 and TNF- α .²⁵

Furthermore, it should be noted that TLR that residing on the cell surface (TLR2, TLR4, TLR5) and that recognize bacterial constituents, induces miRNA-146a/b, while TLRs that detect viral nucleic acids and that are located intracellularly (TLR3, TLR7, and TLR9), have little effect on miRNA-146a/b expression.²⁴

Unlike miRNA-146, miR-155 is highly induced by TLRs that detect viral nucleic acids, and its expression is strongly induced by inflammatory cytokines such as IFN and TNF, concluding that this miRNA is a component of the innate immune response. The action of miR-155 on the regulation of T and B cells has been demonstrated through studies with miR-155 knockout mice, which are immune competent.²⁶

Studies based on the action of miRNAs in the regulation of the innate immune response in monocytes and macrophages analyzed the increase of the expression of miRNAs in the lineage of monocytic cells treated with TLR4 and lipopolysaccharides. The results showed that there was increased expression of some miRNAs, among them miRNA-146 and miR-155.²⁴

In contrast to miR-155 and miR-146, miR-125b is suppressed in mouse macrophages on LPS treatment. The role of miR-125b in macrophages may be to ensure that the LPS pathway is switched off in the absence of microbial infection.²⁷

Regarding adaptive immune response, high expression of miR-150 was identified both during maturation of B and T cells, as well as when those cells are already in the mature phase, but there is no evidence of their occurrence in the progenitors of these cells. Xiao *et al.*²⁸ have demonstrated that the expression miRNA-150 controls several stages of lymphocyte development.²⁸

The expression of various miRNAs (e.g., miR-21, miR-22, miR-24, miR-103, miR-155 and miR-204) is elevated in *in vitro* activated T cells, while miR-16, miR-26, miR-30, miR-150 and miR-181 were suppressed after stimulation.²⁹ Pedersen and David³⁰ reaffirmed the performance of miR-155 and miR-146 as regulators of the innate inflammatory response and also added miR-132 and miR-223, the latter being involved in the regulation of circulating neutrophils, because studies show that its absence leads to an increase in the number of granulocyte progenitors in the bone marrow and an increase in circulating neutrophil maturation, suggesting that it is involved in the negative regulation of maturation but not in the differentiation of granulocytes.³⁰

Based on the experiments of Chen and Lodish,³¹ it has been shown that miR-181 is related to the development of B and T lymphocytes. In this study, the cloning of approximately 100 miRNAs exclusive of the bone marrow of the rat was performed and systematically analyzed its gene expression, identifying miR-181 as one of three miRNAs differentially expressed in rat hematopoietic organs. At a given time, the analysis of miR-181 gene expression was significantly higher in the thymus while there was a decrease in expression in hematopoietic cells, demonstrating that the expression of miR-181 in the other progenitor cells was decreased and more differentiated in B lymphocytes, which suggests a role for this miRNA in the development of T cells as well as in B cells.³¹

Pedersen and David³⁰ also analyzed the performance of this miRNA on T cells in the thymus, and their results were also suggestive for miR-181 performance in the modulation of these cells, thus exerting control of the immune response.³⁰

Studies with miR-16 have shown that it is found in high levels in many cells, including those involved in inflammation, such as monocytes, neutrophils, B cells, T cells.³¹ This expression suggests that miRNA-16 may have a role in preventing cell cycle progression.³² In contrast, miR-147, in mouse macrophages, functioned as a TLR-negative regulator associated with signaling events. The expression of miR-147 was higher after TLR4 cellular activation than TLR2 or TLR3, the data obtained demonstrated that this miRNA might be related to the control of the immunological response, being a macrophage-negative regulator, avoiding excesses in its activation.³²

Periodontal Disease and Immune Response

Although bacterial plaque is the main etiological factor of periodontal disease, the host immune response determines the susceptibility to the disease, since there is a delicate balance between the microorganisms found on the plaque and the response of the host.²²

In healthy individuals, the immune response provides a specific and well-regulated defense, thereby preventing the development of the periodontal disease, whereas, in immunocompromised individuals, an imbalance of this response occurs, thus increasing the susceptibility to disease development.²²

Bacterial components such as LPS, found in the cell wall of Gram-negative bacteria, represent essential and potent stimulators of cell secretion of various cytokines and TLR-mediated growth factors. The binding of LPS to TLR4 induces the production of inflammatory cytokines of the innate immune response, which contribute to tissue destruction.²²

Cytokines and chemokines are involved in the immu-

nopathogenesis of periodontal disease by causing the selective migration of distinct cell types and by promoting the maintenance of specific leukocyte subtypes in periodontal tissues.³³ Numerous researches have demonstrated a relationship between the progression of periodontal disease and the expression of Th1-type cytokines at inflammation sites, such as IL-1, IL-2, IFN- γ and TNF- α , and IL-6, TNF- α , and prostaglandin E2 (PGE2) lipid mediators that stimulate macrophages and induce changes in connective tissue and extracellular matrix.³⁴ However, other studies such as that by Lappin *et al.*³⁵ have demonstrated the involvement of humoral cytokines and the Th2 immune response, being the cells involved in this response much more abundant in periodontal lesions.³⁵

NF- κ B is responsible for initiating the transcription of several cytokines, growth factors and adhesion molecules, such as TNF- α , IL-1, IL-6, IL-8. The host immune system is essential in the disease process since during an infectious process, and its cells are concentrated at the site of infection.³⁶ The inflammatory activity observed in periodontal disease is a process due to migration and cell recruitment, and the establishment of this activity involves the initial displacement and adhesion of the leukocytes to the vascular endothelium, as well as their subsequent emigration to the tissue, processes that comprise several stages and involve adhesion and chemotactic proteins. After crossing the walls of the vessels, responding to the chemotactic stimuli, the leukocytes go to the inflammatory site in which they will perform their effector functions.^{37,38}

miRNA and Periodontal Disease

Although several studies report the role of miRNAs in regulating the innate and adaptive immune response, little is known about the regulation of miRNA during experimental periodontal disease.

Nahid *et al.*³⁸ reported increased expression of miR-146 in a model of polymicrobial periodontitis in mice. The results suggested that miR-146 can directly or indirectly modulate or alter the chronic periodontal pathology induced by these microorganisms since it has already been seen that this miRNA has an activity in the control of the immune response.

More recently, Xie and colleagues³⁹ compared the expression of miRNAs from patients with periodontal inflammation with control individuals by microarray and validation by Real-Time PCR. They demonstrated that in addition to the miR-146 other microRNAs were increased in inflamed gingivae such as miR-155, miR-203 and miR-223 and all of which have putative targets for regulating the inflammatory response. In another cohort study,⁴⁰ confirmed increased expression of miR-155 and miR-223 in patients with periodontal disease and observed increased miR-185 expression in tissues with periodontal disease. This miRNA has already

been described as a regulator of the immune response, mainly increasing the response via TLR and IL-1 receptors. These data suggest that microRNAs may play an important role in the regulation of inflammation in periodontal disease.⁴¹

Periodontal disease does not only have a unique localization in the mouth, but it also has a systemic effect and may be involved in atherosclerosis of the vessels of the heart. Oral pathogens such as *P. gingivalis* and *Streptococci* have the ability to invade endothelial cells of the heart and accelerate plaque growth and induce macrophage invasion.⁴² Recently, it has been shown that oral pathogens are able to induce an inflammatory response verified by the production of IL-6 and TNF- α in human smooth muscle cells (HSMCs). Comparison of the expression of smooth muscle cell microRNAs from healthy donors and cells from atherosclerotic plaques challenged with oral pathogens demonstrated a significant increase in expression, especially of miR-155-5p, which is involved in morphological regulation of the endothelial cell. The expression of miR-155-5p correlated with IL-6 levels, suggesting a potential role for this microRNA in the inflammation of atherosclerosis induced by oral bacteria.⁴³

Discussion

Until now, it is known that the miRNAs participate in regulating the acquired immune response, such as antibody production and release of inflammatory mediators through their impact on the development and differentiation of immune cells.⁷ This contrasts with the activation of the innate immune response that has been shown to induce rapid changes in the expression levels of miRNAs. Assigning specific functions to the miRNAs is not yet possible, because due to the fact they are in large numbers, they often appear to be functionally redundant. Lack of expression of specific miRNAs, such as miR-155, may reduce the magnitude of the immune response, and lead to immunodeficiency. Other miRNA changes, such as lack of miR-146 or overexpression of miR-155, may result in a very intense reaction to infection. Finally, a constant overexpression of the miR-155 or deletion of miR-146a may lead to chronic inflammation.⁴⁴ Some miRNAs are induced by bacterial and viral TLR ligands as well as inflammatory cytokines, and they have as target key elements of the same pathway, thus providing a post-transcriptional mechanism for regulating the inflammatory response of macrophages.⁴⁵

Once the miRNAs have been proven to regulate the immune response during inflammation,²⁵ and since periodontal disease is a chronic inflammation⁴⁵, where the immune response of the host is closely related to its evolution, further studies must be performed to identify which miRNAs are associated with this pathology.

The discovery of miRNAs as post-transcriptional regulators added a stage of complexity to the mechanisms that govern the elucidation of immune system development and function,⁶ it was seen that numerous types of miRNAs could act at various moments of the maturation of the defense cells, working directly on the immune response of the host.

Conclusion

Thus, considering the studies presented in the literature, this is an area where there is space to be explored, and it is believed that the results of new studies, allows obtaining biomarkers that elucidate the mechanisms by which miRNAs can be responsible for the pathophysiology of periodontal disease.

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