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MICRORNAS – REVOLUTIONARY REGULATORS FOR PERIODONTAL DISEASES.

Periodontology

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ABSTRACT

Until a few years ago, the importance of non-protein-coding genes in biology was mainly restricted to RNA (ribonucleic acid)s, however the scientific research community have recently expanded the species of non-coding RNAs(ncRNAs) mainly micro RNAs(miRNAs). These are a group of short (19-24 nucleotides) non coding RNAs which have emerged as key regulators of gene expression at the post transcription level. As a result, miRNAs take part in regular biological processes within the cells and tissues, and are also involved in pathological processes including inflammatory diseases and even cancer. Since periodontitis is an inflammatory disease, there is an anticipated role of miRNAs in the pathobiology of periodontal disease, which could serve as a biomarker for the same. This review focuses on the proposed basic mechanisms of miRNA regulations during inflammatory responses and their potential role as biomarkers for periodontal diseases and therapeutic targets.

KEYWORDS

microRNA, periodontitis, biomarker, therapeutics.

INTRODUCTION

Periodontitis is a chronic polymicrobial, multifactorial oral disease presenting with dysregulated immune & inflammatory responses at the level of connective tissue & bone support surrounding the teeth, leading to tooth loss if left untreated.[1] Immune responses to bacterial products, & the subsequent production of inflammatory cytokines, are of particular importance in the destruction of periodontal tissue[2]. All aspects of this process, are governed by a group of small RNAs called microRNAs (miRNAs) that only recently have emerged as the most important regulators of bone formation, resorption, remodeling, repair, and disease. miRNAs are naturally occurring small, non-coding RNA molecules, which regulate gene expression post-transcriptionally by binding to the complementary sequences in the coding or 3' untranslated region (UTR) of the target messenger RNAs (mRNAs) and occasionally 5' UTR or coding sequences, thus the protein expression is posttranscriptionally repressed either by blocking or inhibiting translation or inducing target mRNA degradation[3].

HISTORY

MiRNA *lin-4*, was first discovered in 1993 by the Ambros and Ruvkun groups in *Caenorhabditis elegans* [4,5] has revolutionized the field of molecular biology, they also found that *lin-14* was post-transcriptionally downregulated through its 3′ untranslated region (UTR) and that *lin-4* had a complementary sequence to that of the 3′ UTR of *lin-14*[4]. Consequently, they anticipated that *lin-4* regulates *lin-14* at the post-transcriptional level[5]. Ever since, miRNAs have been detected in all animal model systems and some species. New miRNAs and their roles in gene regulation are still being discovered[6].

Process of MicroRNA formation

Biogenesis is the process of formation of miRNAS[7,8,9]. During this process, miRNAs are first transcribed as primary transcripts (pri-miRNA) with a 5′-cap and a 3′-polyA tail. Maturation of pri-miRNA into short, 70-nucleotide RNA molecules with stem-loop structures called, pre-miRNAs by **Drosha** ribonuclease which are then transported from the nucleus into the cytoplasm by the Ran transport receptor **Exportin-5**. Pre-miRNAs are further cleaved in the cytoplasm by **Dicer,** an endoribonuclease to form a short, double-stranded miRNA:miRNA duplex which further unwound into mature miRNA and miRNA single strands by a helicase. The mature miRNAs are then incorporated into the RNA-induced silencing complex (RISC), which initiates RNAi-based gene silencing.[10]

Most miRNAs affect gene expression via gene silencing mechanisms, including mRNA cleavage and translation repression through the RISC[11,12]. The type of silencing mechanism employed by an individual miRNA appears to depend on the level of complementarity between the miRNA and mRNA target[7]. However, these 2 mechanisms are distinguished by the reversibility of their effect on messenger RNA (mRNA): mRNA decay is an irreversible process, while translation inhibition is reversible because stable mRNA can be translated following elimination of translation repression[13,14]. While the majority of miRNAs function via silencing mechanisms, a number of miRNAs are also involved in the upregulation of gene expression. Moreover, miRNAs can both regulate and be regulated by target interactions, suggesting that miRNAs play multiple roles in complex physiological interactions and disease processes[15].

Anticipated mechanisms for miRNA function

1. Deadenylation and destabilization : Removal of poly(A) tail by deadenylases causes destabilization and degradation of mRNA [16,17,18].
2. mRNA cleavage : Argonaute (Ago) - mediated endonucleotic cleavage of target mRNA can occur when the miRNA sequence is complementary to the target gene – binding site[19].
3. Inhibition of Translation initiation : Translation initiation inhibited by miRISC interactions with eukaryotic translation initiation factors (elFs)[20] .
4. Inhibition of translation postinitiation : miRNAs can inhibit translation after initiation during elongation of the nascent peptide [21].
5. Sequestration of mRNA in P- bodies: miRNA bound mRNA can be sequestered from the translational machinery in P – bodies and react with enzymes for 5'- cap removal which further prevents the translation [22,23].
6. miRNA- mediated translational activation : miRNAs are also able to function as translational activators for some of the genes [24].

Control of mechanisms of miRNAs

Regulatory mechanisms of miRNAs are controlled under physiological conditions like regulating protein – encoding genes, which can occur at the level of post transcriptional miRNA expression. Dysfunction of these mechanisms associated with the diseases can result in various genetic and epigenetic alterations that may lead to aberrant expression of miRNAs.

a. Amplification of miRNA-encoding regions[25]
b. Deletion of miRNA-encoding regions[26]
c. Mutations in miRNA sequence (Single nucleotide polymorphisms)[27]
d. Translocation occurring between miRNA-encoding regions and gene promoter regions[28]
e. Epigenetic regulation/ mechanisms such as DNA methylation[29] and Histone modification of promoter regions of miRNAs that can silence miRNA expression[30,31]

In periodontal diseases, anabolic and catabolic processes balance is distressed, affecting the osteoblasts and periodontal progenitors which leads to increased activity of osteoclasts, resulting in increased bone resorption and decreased new bone formation[32]. Osteoclast activations are triggered by lipopolysaccharides (LPS) from bacterial cell walls. This will affect the proinflammatory cytokines such as interleukin IL-6, (IL)–1β, tumor necrosis factor(TNF)-α, stimulating the receptor activator of nuclear factor kappa B - ligand (RANKL) on the osteoclast cell surface and its nuclear target NF-κB, for the differentiation of osteoclast precursors into multinucleated osteoclasts. The expression of these miRNAs was upregulated. A number of miRNAs expressed in periodontal tissues, including miR-34a[33], miR-125a[34], miR-146a[35], miR-223[36], and miR503[37], inhibit osteoclast differentiation. during osteoclastogenesis to provide negative feedback loops related to osteoclast differentiation.

miRNAs as potential clinically useful molecules

Although there is vast evidence that miRNAs play a fundamental role in the pathogenesis of many diseases, the answer for the question, "Can miRNAs be used as novel biomarkers?" remains obscure.

Apoptosis and necrotic cell death results in release of nucleic acids including miRNAs into the blood circulation in a highly stable form and are protected from RNase digestion. This particular characteristic of miRNAs has made interest in the use of miRNAs as non invasive biomarkers of diseases. miRNAs also can be detected in many other biological fluids like, serum, plasma, urine, tears, cerebrospinal fluids, breast milk and saliva. Detection of these non- invasive circulating miRNAs has shown advantage over the gold standard invasive biopsy procedures which are proved to be risky especially in case of cancer patients.[38]

Several miRNA studies related to periodontal diseases have been conducted to compare miRNA expression profiles between healthy and diseased gingival tissues. These observations show that chronic periodontitis is able to modulate the expression of miRNAs in the periodontal tissue.[39]

An experimental periodontal disease study done in $ApoE^{-/-}$ mice explored the expression of miRNAs (miR-146a, miR-132, and miR-155) in maxillas (periodontium) and spleens of $ApoE^{-/-}$ mice infected with P. gingivalis, T. denticola, and T. forsythia as a polymicrobial infection followed by mRNA expression levels of the inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β). However miR-146a had a negative correlation with TNF-α secretion in vitro, reducing levels of the adaptor kinases IL-1 receptor associated kinase 1 (IRAK-1) and TNF receptor-associated factor 6 (TRAF6). The study concluded that miR-146a is upregulated in localized as well as systemic manners during periodontal pathogen infections, where miR-146a may contribute to the control of the resulting periodontal inflammation[40].

The first study report of periodontitis-related miRNA[41] in humans, using miRNA microarray and real-time PCR, reveals that miR-181b, miR-19b, miR-23a, miR-30a, miR-let7a and miR-301a were up-regulated in the inflamed periodontal tissue of moderate-to-advanced chronic periodontitis patients. This study indicated that six miRNAs up-regulated in periodontitis gingiva may play a key role in chronic periodontitis. At the same time, another study[42] investigated the properties of miRNAs in periodontal inflamed and healthy gingival tissues. Twelve selected inflammatory-related miRNAs, miR-126, miR-20a, miR-142-3p, miR-19a, let-7f, miR-203, miR-17, miR-223, miR-146b, miR-146a, miR-155, and miR-205 showed comparable expression levels by microarray and real-time quantitative RT-PCR analyses. Further the target gene scan, predicted the targets of three miRNAs (miR-146a, miR-146b, and miR-155), which showed that these miRNAs have a functional role in TLR- related regulation in inflammatory diseases.

The first study to examine concurrently the expression of miRNAs and mRNAs in healthy and diseased gingival tissues using a whole genome microarrays in a large scale and provides the groundwork to analyze the role of miRNAs in gingival tissue homeostasis and pathology. The expression of 1,205 miRNAs were confirmed by quantitative RT-PCR. One hundred fifty-nine miRNAs were significantly expressed between healthy and diseased gingiva. Four miRNAs (miR-451, miR-223, miR-486-5p, miR-3917) were significantly overexpressed, and 7 (miR-1246, miR-1260, miR-141, miR-1260b, miR-203, miR-210, miR-205) were underexpressed by > 2-fold in diseased vs. healthy gingiva. Sixty enriched miRNA gene sets with target genes involved were identified by Gene Set Enrichment Analysis (GSEA)[43].

In a similar study a total RNAs were isolated from inflamed and non inflamed gingival tissues from Japanese patients to examine and compare the miRNA expression profiles using microarray analysis. miRNA targets were identified by Ingenuity Pathways Analysis (IPA) and the TargetScan databases. In inflamed gingiva, 17 overexpressed and 22 underexpressed miRNAs were identified. The three most overexpressed miRNAs were miR-150, miR-223, and miR-200b, and the three most underexpressed miRNAs were miR-379, miR-199a-5p, and miR-214 in inflamed gingiva. The findings suggest a relationship between miRNAs and periodontitis in periodontal tissue destruction[44].

The above discussed are the five classic and basic studies done to demonstrate the role of miRNAs in periodontal pathology.

To explore possible ways of miR-146 contribution to the pathogenesis of periodontitis, by using RT- PCR in *Porphyromonas gingivalis (p.g)* lipopolysaccharide (LPS) - stimulated human gingival fibroblasts (HGFs) were transfected with miR-146a &miR-146b-5p inhibitors. Results showed an increase in pro-inflammatory cytokines such as IL-β, IL-6 & TNF-α through interleukin – 1 receptor - associated kinase 1 (IRAK1) activation[45].

The expression levels of miR-146a and its targets, including TNF-α, IL-1β, and IL-6, were evaluated in gingival tissue of human healthy individulas and with chronic periodontitis (CP) patients. Higher levels of miR-146a and lower levels of TNF-α and IL-6 were seen in CP patients than healthy individuals. A positive correlation was found with miR-146a levels, probing depth and clinical attachment loss suggesting that miR-146a may influence the pathogenesis of periodontitis[46]. One of the recent study[47] determined whether miRNA-146a expression has any potential link in gingival tissues of generalized aggressive periodontitis (GAgP) patients and its association with disease severity. This assessment found a 17.8-fold elevation in miR-146a expression in GAgP patients when compare to healthy subjects.

Expression of miR-128, miR-34a, miR-381[48] and miR-144-5p[49] were also found to be upregulated in gingival tissues from chronic periodontitis patients. The first study performed on miRNA expression profiles comparing CP and AgP in gingival tissue samples. When miRNA microarrays compared the expression of 754 inflammatory-related miRNAs, similar miRNA expression profiles from CP and AgP groups were presented. The most overexpressed miRNAs in both groups were miR-1274b, let-7b-5p, miR-24-3p, miR-19b-3p, miR-720, miR-126-3p, miR-17-3p and miR-21-3p.

On the other hand, an increase in gingival crevicular fluid (GCF) flow is observed during gingival and periodontal inflammation should be considered. Accordingly, increased amount of miRNA might pass the junctional epithelium, thus may arrive in GCF and saliva[50]. Very few studies correlated the relative expression of miRNAs found in GCF and saliva in chronic periodontitis patients. The first study[51] to verify the presence of miRNAs in GCF, discovered that GCF from periodontitis patients has unique miRNA profiles. When compared the differentially expressed 40 miRNAs in GCF to those previously identified in blood and gingival tissue samples of periodontitis, Up regulated miRNA and down-regulated miRNA sets determined in different sample types, and it was noted that they are mostly distinctive. High expression levels of miR-223-3p, miR-203a and miR-205-5p in GCF samples of periodontitis patients were confirmed. Very recently the expression of Salivary miR-381-3p[50] and miR-143-3p[52] was correlated with periodontitis condition in chronic periodontitis patients.

The effects of periodontitis on serum miRNAs profile in a rat model was investigated under four groups. The control groups received no treatment for 2 or 4 weeks. In the other two groups, periodontitis was ligature induced for 2 or 4 weeks. Serum levels of miR-207, miR-376-3p, and miR-495 were higher in the periodontitis group than in the control group. These suggest that periodontitis could contribute to the increased miRNAs expressions in serum[53]. Recently a case-control study[54] was done to assess the association between human serum miRNAs in periodontitis patients. miR-664a-3p, miR-501-5p, miR-21-3p in serum were expressed at higher levels in the periodontitis group compared to the control group, suggesting miRNAs in blood may thus serve as biomarkers of periodontitis and may increase the understanding of the periodontal systemic connection.

Several miRNA specific methods from identification of candidate miRNAs up to influencing biological processes by their modulation have been developed. **Firstly**, for high throughput screening of miRNAs and generation of differential microRNA profiles in a particular experimental setting, miRNA array technologies are used. A **second** confirming array technology helps to narrow down the number of false positive candidate miRNAs. Further validation of miRNA arrays is usually done by qRT-PCR of single miRNAs. MicroRNA target genes may then be identified by using computational methods.

miRNA based therapeutic approaches

It is assumed that miRNAs regulate >100 target genes at the same time, whereas regulation of any given gene can be mediated by several miRNAs. Therefore more than one miRNA is needed for any successful therapeutic intervention. Thus the detailed functional analysis of any given miRNA in its pathological setting is needed before any intervention. In this context it is noted that certain miRNAs appear to be relevant in several pathologies. It is likely that these small powerful regulators hold great potential for the treatment of periodontal diseases also.

There are 2 major strategies to therapeutically modulate dysregulated miRNAs in disease[55]:

1. Using miRNA **mimics** to restore physiological levels of miRNAs that are down regulated (ex: tumor suppressor miRNAs, such as *let-7, miR-34*)
2. The use of miR **inhibitors** targeted against overexpressed miRNAs (ex: oncomirs, such as miR-21 or miR-155).

For **example**, miRNA therapeutics may be applied to restore the function of lost or downregulated miRNAs related to osteoblastogenesis or to inhibit the function of upregulated miRNAs related to osteoclast differentiation and function. In recent years, several different miRNA delivery systems have been developed, including viral and nonviral approaches[10].

Viral miRNA delivery systems are based on **retroviruses, lentiviruses, and adenoviruses** and are distinguished by a high infection efficiency and high miRNA or antagomir expression levels, while suffering from relatively higher toxicity and immunogenicity levels.

Nonviral miRNA delivery approaches include **lipid-based delivery systems** such as liposomes as well as **polymer-based approaches** including polyethylenimine (PEI), poly(lactide-co-glycolide) (PLGA), and poly(amidoamine) (PAMAM) dendrimers

Other recently developed **miRNA carriers** include chitosan, protamine and collagen , as well as gold-, iron-, and silica-based nanoparticles.

Novel aptamer-miRNA conjugates have been developed to deliver miRNAs into specific cell types, resulting in a reduction of the dose required for pharmacological effects, a reduction in OTEs (off-target effects) and reduced toxicity.

Novel plasmid-based miRNA inhibitor system (PMIS) has been developed that relies on specific binding of a PMIS molecule to target miRNAs, resulting in the introduction of a new secondary structure and the formation of a stable PMIS-miR complex.

Future directions:

At present the couple of studies are at the preclinical stage. The major hurdle to be overcome in order to translate these results into the clinic include the effective targeting of therapy (ex. Tissue-specific delivery, dosage and pharmacodynamics) and safety concerns (ex. Off-target effects (OTEs), RNA mediated immunostimulation and the use of viral vectors).

This is an area very much still in infancy that is almost certain to flourish in the near future as the field matures, and promises to add to the current weapon of therapies available to the clinician in the continual fight against disease.

In conclusion, miRNAs show their greatest, and certainly most immediate potential, as novel biomarkers of diagnosis, prognosis and as predictors of treatment response. Although it is clear that the functional importance of miRNAs in medicine is gaining momentum rapidly, it is equally obvious that we still have much to learn from these tiny molecules.

REFERENCES:

1. Kalea, A. Z., Hoteit, R., Suvan, J., Lovering, R. C., Palmen, J., Cooper, J. A., Khodiyar, V. K., Harrington, Z., Humphries, S. E., & D'Aiuto, F. (2015). Upregulation of gingival tissue miR-200b in obese periodontitis subjects. *Journal of Dental Research*, *94*(3)(Suppl.), 59S–69S. https://doi.org/10.1177/0022034514568197
2. Ogata, Y., Matsui, S., Kato, A., Zhou, L., Nakayama, Y., & Takai, H. (2014). MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients. *Journal of Oral Science*, *56*(4), 253–260. https://doi.org/10.2334/josnusd.56.253
3. Lee, Y. H., Na, H. S., Jeong, S. Y., Jeong, S. H., Park, H. R., & Chung, J. (2011). Comparison of inflammatory microRNA expression in healthy and periodontitis tissues. *Biocell*, *35*(2), 43–49. https://doi.org/10.32604/biocell.2011.35.043
4. Lee, R. C., Feinbaum, R. L., Ambros, V., & The, C. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, *75*(5), 843–854. https://doi.org/10.1016/0092-8674(93)90529-Y
5. Wightman, B., Ha, I., & Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell*, *75*(5), 855–862. https://doi.org/10.1016/0092-8674(93)90530-4
6. O'Brien, J., Hayder, H., Zayed, Y., & Peng, C. (2018). Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in Endocrinology*, *9*, 402. https://doi.org/10.3389/fendo.2018.00402
7. Bartel, D. P. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*, *116*(2), 281–297. https://doi.org/10.1016/s0092-8674(04)00045-5
8. Carthew, R. W., & Sontheimer, E. J. (2009). Origins and mechanisms of miRNAs and siRNAs. *Cell*, *136*(4), 642–655. https://doi.org/10.1016/j.cell.2009.01.035
9. Cech, T. R., & Steitz, J. A. (2014). The noncoding RNA revolution-trashing old rules to forge new ones. *Cell*, *157*(1), 77–94. https://doi.org/10.1016/j.cell.2014.03.008
10. Luan, X., Zhou, X., Trombetta-eSilva, J., Francis, M., Gaharwar, A. K., Atsawasuwan, P., & Diekwisch, T. G. H., Atsawasuwan P et al. (2017) MicroRNAs and Periodontal Homeostasis. *Journal of Dental Research*, 96(5), 491–500. https:// doi. org/ 10. 1177/ 0022034516685711
11. Hofacker, I. L. (2007). How microRNAs choose their targets. *Nature Genetics*, *39*(10), 1191–1192. https://doi.org/10.1038/ng1007-1191
12. MacFarlane, L. A., & Murphy, P. R. (2010). MicroRNA: Biogenesis, function and role in cancer. *Current Genomics*, *11*(7), 537–561. https:// doi. org/ 10. 2174/ 138920210793175895
13. Maroney, P. A., Yu, Y., Fisher, J., & Nilsen, T. W. (2006). Evidence that microRNAs are associated with translating messenger RNAs in human cells. *Nature Structural and Molecular Biology*, *13*(12), 1102–1107. https://doi.org/10.1038/nsmb1174
14. Valencia-Sanchez, M. A., Liu, J., Hannon, G. J., & Parker, R. (2006). Control of translation and mRNA degradation by miRNAs and siRNAs. *Genes and Development*, *20*(5), 515–524. https://doi.org/10.1101/gad.1399806
15. Orang, A., Safaralizadeh, R., & Kazemzadeh-Bavili, M. (2014). Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. *International Journal of Genomics*. PubMed: 970607
16. Giraldez, A. J., Mishima, Y., Rihel, J., Grocock, R. J., Van Dongen, S., Inoue, K., Enright, A. J., & Schier, A. F. (2006) Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science*, *312*(5770), 75–79. https:// doi. org/ 10. 1126/ science. 1122689
17. Huntzinger, E., & Izaurralde, E. (2011). Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. *Nature Reviews. Genetics*, *12*(2), 99–110. https://doi.org/10.1038/nrg2936
18. Wu, L., Fan, J., & Belasco, J. G. (2006). MicroRNAs direct rapid deadenylation of mRNA. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(11), 4034–4039. https://doi.org/10.1073/pnas.0510928103
19. Yekta, S., Shih, I. H., & Bartel, D. P. (2004). microRNA-directed cleavage of HOXB8 mRNA. *Science*, *304*(5670), 594–596. https://doi.org/10.1126/science.1097434
20. Humphreys, D. T., Westman, B. J., Martin, D. I., & Preiss, T. (2005). MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(47), 16961–16966. https://doi.org/10.1073/pnas.0506482102
21. Olsen, P. H., & Ambros, V. (1999). The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking LIN-14 protein synthesis after the initiation of translation. *Developmental Biology*, *216*(2), 671–680. https:// doi. org/1 0. 1006/ dbio.1999.9523
22. Liu, J. MA, Valencia-Sanchez, M. A., Hannon, G. J., & Parker, R. (2005) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nature Cell Biology*, *7*(7), 719–723. https://doi.org/10.1038/ncb1274
23. Sen, G. L., & Blau, H. M. (2005). Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nature Cell Biology*, *7*(6), 633–636. https://doi.org/10.1038/ncb1265
24. Vasudevan, S., Tong, Y., & Steitz, J. A. (2007). Switching from repression to activation: microRNAs can up-regulate translation. *Science*, *318*(5858), 1931–1934. https://doi.org/10.1126/science.1149460
25. Huse, J. T., Brennan, C., Hambardzumyan, D., Wee, B., Pena, J., Rouhanifard, S. H., Sohn-Lee, C., Le Sage, C., Agami, R., Tuschl, T., & Holland, E. C. (2009). The PTEN—Regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. *Genes and Development*, *23*(11), 1327–1337. https://doi.org/10.1101/gad.1777409
26. Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F., & Croce, C. M. (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(24), 15524–15529. https://doi.org/10.1073/pnas.242606799
27. Li, W., Duan, R., Kooy, F., Sherman, S. L., Zhou, W., & Jin, P. (2009) Germline mutation of microRNA-125a is associated with breast cancer. *Journal of Medical Genetics*, *46*(5), 358–360. https://doi.org/10.1136/jmg.2008.063123
28. Bousquet, M., Quelen, C., Rosati, R., Mansat-De Mas, V., La Starza, R., Bastard, C., Lippert, E., Talmant, P., Lafage-Pochitaloff, M., Leroux, D., Gervais, C., Viguié, F., Lai, J. L., Terre, C., Beverlo, B., Sambani, C., Hagemeijer, A., Marynen, P., Delsol, G., . . . Brousset, P. (2008). Myeloid cell differentiation arrest by miR -125b-1 in myelodysplastic syndrome and acute myeloid luekemia with the t (2; 11)(p21;q23) translocation. *Journal of Experimental Medicine*, *205*(11), 2499–2506. https:// doi. org/ 10. 1084/jem.20080285
29. Esteller, M. (2008). Epigenetics in cancer. *New England Journal of Medicine*, *358*(11), 1148–1159. https://doi.org/10.1056/NEJMra072067
30. Weber, B., Stresemann, C., Brueckner, B., & Lyko, F. (2007). Methylation of human microRNA genes in normal and neoplastic cells. *Cell Cycle*, *6*(9), 1001–1005. https://doi.org/10.4161/cc.6.9.4209
31. Bueno, M. J., Pérez de Castro, I., Gómez de Cedrón, M., Santos, J., Calin, G. A., Cigudosa, J. C., Croce, C. M., Fernández-Piqueras, J., & Malumbres, M. (2008). Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR –ABL1 oncogene expression. *Cancer Cell*, *13*(6), 496–506. https:// doi. org/ 10. 1016/ j. ccr. 2008. 04.018
32. Redlich, K., & Smolen, J. S. (2012). Inflammatory bone loss: Pathogenesis and therapeutic intervention. *Nature Reviews. Drug Discovery*, *11*(3), 234–250. https://doi.org/10.1038/nrd3669
33. Chen, L., Holmstrøm, K., Qiu, W., Ditzel, N., Shi, K., Hokland, L., & Kassem, M. (2014). MicroRNA-34a inhibits osteoblast differentiation and in vivo bone formation of human stromal stem cells. *Stem Cells*, *32*(4), 902–912. https:// doi. org/ 10. 1002/ stem. 1615
34. Guo, L. J., Liao, L., Yang, L., Li, Y., & Jiang, T. J. (2014). MiR-125a TNF receptor-

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associated factor 6 to inhibit osteoclastogenesis. *Experimental Cell Research*, *321*(2), 142– 152. https://doi.org/10.1016/j.yexcr.2013.12.001

35. Nakasa, T., Shibuya, H., Nagata, Y., Niimoto, T., & Ochi, M. (2011). The inhibitory effect of microRNA-146a expression on bone destruction in collagen induced arthritis. *Arthritis and Rheumatism*, *63*(6), 1582–1590. https://doi.org/10.1002/art.30321
36. Sugatani, T., & Hruska, K. A. (2007). MicroRNA-223 is a key factor in osteoclast differentiation. *Journal of Cellular Biochemistry*, *101*(4), 996–999. https:// doi. org/ 10. 1002/ jcb.21335
37. Chen, C., Cheng, P., Xie, H., Zhou, H. D., Wu, X. P., Liao, E. Y., & Luo, X. H. (2014). MiR503 regulates osteoclastogenesis via targeting RANK. *Journal of Bone and Mineral Research*, *29*(2), 338–347. https://doi.org/10.1002/jbmr.2032
38. Weber, J. A., Baxter, D. H., Zhang, S., Huang, D. Y., Huang, K. H., Lee, M. J., Galas, D. J., & Wang, K. (2010). The microRNA spectrum in 12 body fluids. *Clinical Chemistry*, *56*(11), 1733–1741. https://doi.org/10.1373/clinchem.2010.147405
39. Fujimori, K., Yoneda, T., Tomofuji, T., Ekuni, D., Azuma, T., Maruyama, T., Mizuno, H., Sugiura, Y., & Morita, M. (2019). Detection of salivary miRNAs reflecting chronic periodontitis: A pilot study. *Molecules*, *24*(6), 1034. https:// doi. org/ 10. 3390/ molecules 24061034
40. Nahid, M. A., Rivera, M., Lucas, A., Chan, E. K., & Kesavalu, L. (2011). Polymicrobial infection with periodontal pathogens specifically enhances microRNA miR-146a in ApoE−/− mice during experimental periodontal disease. *Infection and Immunity*, *79*(4), 1597–1605. https://doi.org/10.1128/IAI.01062-10
41. Lee, Y. H., Hee, S. N., Jeong, S. Y., Jeong, S. H., Park, H. R., & Chung, J. (2011). Comparison of inflammatory microRNA expression in healthy and periodontitis tissues. *Biocell*, *35*, 42–49.
42. Xie, Y. F., Shu, R., Jiang, S. Y., Liu, D. L., & Zhang, X. L. (2011). Comparison of microRNA profiles of human periodontal diseased and healthy gingival tissues. *International Journal of Oral Science*, *3*(3), 125–134. https:// doi. org/ 10. 4248/ IJO S11046
43. Stoecklin-Wasmer, C., Guarnieri, P., Celenti, R., Demmer, R. T., Kebschull, M., & Papapanou, P. N. (2012). MicroRNAs and their target genes in gingival tissues. *Journal of Dental Research*, *91*(10), 934–940. https://doi.org/10.1177/0022034512456551
44. Ogata, Y., Matsui, S., Kato, A., Zhou, L., Nakayama, Y., & Takai, H. (2014). MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients. *Journal of Oral Science*, *56*(4), 253–260. https://doi.org/10.2334/josnusd.56.253
45. Xie, Y. F., Shu, R., Jiang, S. Y., Liu, D. L., Ni, J., & Zhang, X. L. (2013). MicroRNA-146 inhibits pro-inflammatory cytokine secretion through IL-1 receptor-associated kinase 1 in human gingival fibroblasts. *Journal of Inflammation*, *10*(1), 20. https:// doi.o rg/ 10. 1186/ 1476-9255-10-20
46. Motedayyen, H., Ghotloo, S., Saffari, M., Sattari, M., & Amid, R. (December 2015). Evaluation of MicroRNA-146a and its targets in gingival tissues of patients with chronic periodontitis. *Journal of Periodontology*, *86*(12), 1380–1385. https:// doi. org/ 10. 1902/ jop. 2015.150319
47. Ghotloo, S., Motedayyan, H., Safari, A. D., & Sattari, M. M. (2018). Assessment of microRNA-146a in generalized aggressive periodontitis and its association with disease severity. *Journal of Periodontal Research*;1–6
48. Na, H. S., Park, M. H., Song, Y. R., Kim, S., Kim, H. J., Lee, J. Y., Choi, J. I., & Chung, J. (September 2016). Elevated MicroRNA-128 in periodontitis mitigates tumor necrosis factor-α response via p38 signaling pathway in macrophages. *Journal of Periodontology*, *87*(9), e173–e182. https://doi.org/10.1902/jop.2016.160033
49. Li, J., Wang, R., Ge, Y., Chen, D., Wu, B., & Fang, F. (2019). Assessment of microRNA-144-5p and its putative targets in inflamed gingival from chronic periodontitis patients. *Journal of Periodontal Research*, *54*(3), 266–277. https://doi.org/10.1111/jre.12627
50. Schmalz, G., Li, S., Burkhardt, R., Rinke, S., Krause, F., Haak, R., & Ziebolz, D. (2016). MicroRNAs as salivary markers for periodontal diseases: A new diagnostic approach? *BioMed Research International*, *2016*, 1027525. https:// doi. org/ 10. 1155/ 2016/ 1027525. Epub June 27 2016. PubMed: 27429973, PubMed Central: PMC4939343
51. Saito, A., Horie, M., Ejiri, K., Aoki, A., Katagiri, S., Maekawa, S. et al. (2017). MicroRNA profiling in gingival crevicular fluid of periodontitis-a pilot study. *FEBS Open Bio*, *7*(7), 981–994. https://doi.org/10.1002/2211- 5463.12238 PMID: 28680811.
52. Nisha, K. J., Janam, P., & Harshakumar, K. (2019 October). Identification of a novel salivary biomarker miR-143-3p for periodontal diagnosis: A proof of concept study. *Journal of Periodontology*, *90*(10), 1149–1159. https://doi.org/10.1002/JPER.18-0729. Epub May 10 2019. PubMed: 31021403
53. Tomofuji, T., Yoneda, T., Machida, T., Ekuni, D., Azuma, T., Kataoka, K., Maruyama, T., & Morita, M. (2016 May). MicroRNAs as serum biomarkers for periodontitis. *Journal of Clinical Periodontology*, *43*(5), 418–425. https://doi.org/10.1111/jcpe.12536. Epub April 18 2016. PubMed: 26910654
54. Yoneda, T., Tomofuji, T., Ekuni, D., Azuma, T., Maruyama, T., Fujimori, K., Sugiura, Y., & Morita, M. (2019 May). Serum microRNAs and chronic periodontitis: A case-control study. *Archives of Oral Biology*, *101*, 57–63. https:// doi. org/ 10. 1016/ j. archoralbio. 2019. 03.009. Epub March 13 2019. PubMed: 30889506
55. Bui, T. V., & Mendell, J. T. (2010 June 1). Myc: Maestro of microRNAs. *Genes and Cancer*, *1*(6), 568–575. https://doi.org/10.1177/1947601910377491, PubMed: 20882107, PubMed Central: PMC2946068