The Role of Noncoding RNAs in Osteogenic Differentiation of Human Periodontal Ligament Stem Cells

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Abstract
Chronic inflammatory diseases, including periodontitis, are the most common causes of bone tissue destruction. Periodontitis often leads to loss of connective tissue homeostasis and reduced alveolar bone levels. Human periodontal ligament stem cells (PDLSCs), a population of multipotent stem cells derived from periodontal ligament tissues, are considered as candidate cells for the regeneration of alveolar bone and periodontal tissues. Periodontitis impairs the osteogenic differentiation of human PDLSCs. Noncoding RNAs (ncRNAs), including long noncoding RNA (lncRNA), microRNA (miRNA), and circular RNA (circRNA), have been proposed as vital regulators influencing several differentiation processes including bone regeneration. Still, the molecular mechanisms of ncRNAs regulating osteogenic differentiation of human PDLSCs remain poorly understood. Exploring the influence of ncRNAs in the process of osteogenic differentiation of human PDLSCs may provide novel therapeutic strategies for tissue regeneration as the regeneration of the lost periodontium is the ultimate goal of periodontal therapy.

Keywords
PDLSC, periodontitis, noncoding RNA, miRNA, lncRNA, circRNA

Received: 25 January 2021; reviewed: 25 January 2021; accepted: 5 February 2021

Introduction
Periodontal diseases, including gingivitis and periodontitis, are possibly the most common diseases in humans.¹ Periodontitis is a chronic non-communicable disease (NCD) that shares risk factors and social determinants with the major NCDs that cause approximately two-thirds of deaths including diabetes, heart diseases, chronic respiratory disease, and cancer.² According to the recent Global Burden of Disease Study (GBD, 1990–2010), severe periodontitis is the sixth most prevalent disease globally, with approximately 743 million people affected and an overall prevalence of 11.2%.³,⁴ Individuals suffering from periodontitis are at risk of losing multiple teeth leading to edentulism and masticatory dysfunction, hence affecting their nutrition, self-esteem, and quality of life, along with imposing huge socio-economic impacts and healthcare costs.⁵,⁶ Periodontal diseases are accountable for 3.5 million years lived with disability.⁷ As a result of severe periodontitis alone, the global cost of lost productivity has been estimated to be 54 billion USD per year.⁸ The prevalence of periodontitis tends to increase with age, and the incidence escalates steeply in adults aged between 30 and 40 years. This burden will continue to rise with the increasing aging population due to increased tooth retention worldwide.⁹,¹⁰

The periodontal ligament (PDL) is a non-mineralized connective tissue that holds the tooth in place by attaching cementum to the inner wall of the alveolar bone socket.¹¹

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Human PDL contains stem cells that are capable to differentiate into osteoblasts, cementoblasts, and adipocytes.\(^{12,13}\) Because of these properties, the periodontal mesenchymal stem cells (PDLSCs) were considered to be an ideal source in the repair, regeneration, and maintenance of alveolar bone.\(^{14-16}\) However, several factors in the microenvironment, such as aging\(^{17}\) and inflammation,\(^{18}\) might cause dysfunction of PDLSCs. As a consequence, their osteogenic capacity might be greatly inhibited.\(^{19}\) Chronic periodontitis is considered to be caused by bacterial infection and related host immunological responses, resulting in the destruction of alveolar bone and connective tissue.\(^{19}\) Although researchers report that inflammation inhibits the osteogenic ability of PDLSCs, the exact mechanisms remain unclear.\(^{18,20}\)

Noncoding RNAs (ncRNAs) are usually considered not to possess protein-coding capabilities, rather they mainly function as key gene expression and protein regulators to govern physiological and pathological processes.\(^{21}\) Different cellular processes are regulated by an array of ncRNAs, which actively contribute to lineage specification.\(^{22}\) Micro-RNAs (miRNAs) are the most studied type of ncRNA, and their role in stem cell maintenance and differentiation has been widely explored.\(^{23-25}\) Long noncoding RNAs (lncRNAs) are another type of ncRNA with a length of >200 nucleotides. Substantial evidence demonstrates that some lncRNAs, including MEG3, H19, and lncRNA-ANCR, could govern osteogenic differentiation of stem cells under physiological and pathological conditions.\(^{26-28}\) Circular RNA (circRNA) is a recently identified type of lncRNA and its role in several biological processes is becoming more and more evident.\(^{22}\)

| ncRNA type | Length | Precursor | Main RNA modification types | Structure | Half-life | Main function | Ref |
|------------|--------|-----------|-----------------------------|-----------|----------|--------------|-----|
| miRNA      | 21-25 nt | pri-miRNA or pre-miRNA | 5′-phosphomethylation (5′-Pme\(_{2}\)) 3′ Uridylation adenose to inosine transition (A-to-I) N6-methyladenosine (m6A) | Stem-loop | Variable from <1 h to >12 h | Repression of gene expression | 29,30 |
| IncRNA     | >200 nt | Genic and intergenic regions, promoters, and enhancers | N6-methyladenosine (m6A) N1-methyladenosine (mA1) 5-methylcytosine (m5C) pseudouridine (Ψ) adenose to inosine transition (A-to-I) | Complex secondary and tertiary structures | Variable from <2 h to >16 h | Gene expression regulation through interaction with DNA, RNA, and proteins | 31,32 |
| circRNA    | Combination of 1+ exons/ introns | Transcribed RNA sequences (including pre-mRNA and lncRNA). | N6-methyladenosine (m6A) | Circular | Usually long-lived (>48 h) | miRNA/protein sequestration | 33-35 |

A comparison of the main features of the 3 different ncRNA types are reported in Table 1.

There is a growing body of literature on the role of ncRNAs in human PDLSCs (PDLSCs). It is imperative to comprehend the molecular mechanisms of osteogenic differentiation of human PDLSCs and how to tune it to be able to construct and replace periodontal tissues (Figure 1). The aim of this review is therefore to provide an overview on the role of ncRNA in the regulation of osteogenic differentiation of human PDLSCs.

### Expression and Function of miRNA in Osteogenic Differentiation of Human PDLSCs

MiRNAs represent a subset of small ncRNAs (~22 nucleotides in length on average) that negatively regulate the expression of target genes and play a vital role in cell differentiation.\(^{12,36,37}\) As a general mechanism, they are able to inhibit mRNA translation by binding to the 3′ untranslated regions (3′-UTR) of target genes and promote mRNA degradation (Figure 2).\(^{38}\) Therefore, they play a crucial part in diverse biological processes, including but not limited to development, cell differentiation, proliferation, and apoptosis.\(^{39}\) Numerous miRNAs, such as components of the miR-30 family,\(^{40}\) have been reported to regulate the osteogenesis of human mesenchymal stem cells. Recently, miRNAs have also been shown to be involved in periodontal diseases,\(^{41,42}\) and some were investigated as potential salivary biomarkers for periodontal disease and other oral pathologies.\(^{43}\)
Different miRNAs have been identified as playing a role in the regulation of osteogenesis of human PDLSCs. Table 2 summarizes the main findings and focuses on studies that identified both the miRNAs and their target genes/pathways, thus providing a comprehensive explanation of their mechanisms of action.

While some miRNAs appear to have a clearly defined role in the promotion or inhibition of osteogenesis, at least 2 of them show a context-dependent type of activation or effect on differentiation. In particular, miR-17 seems to inhibit osteogenic differentiation of healthy human PDLSCs, but it has a promoting effect when the cells are isolated from periodontitis-affected patients or cultured under inflammatory conditions. Similarly, miR-21 shows different effects depending on whether the differentiation potential of PDLSCs is investigated under mechanical stimulation or during inflammation. These results highlight the complexity of gene expression regulation in...
Table 2. miRNA Involved in the Regulation of Osteogenic Differentiation of Human Periodontal Ligament Stem Cells (PDLSC).

| miRNA | Target gene | miRNA regulation in human PDLSCs | Role of miRNA in osteogenic differentiation of human PDLSCs | Translational relevance | Ref |
|-------|-------------|---------------------------------|----------------------------------------------------------|------------------------|-----|
| miR-17 | SMURF1      | ↑ during differentiation and ↓↓ in periodontitis | Context-dependent role. Inhibitory effect in healthy PDLSC, promotes osteogenesis in periodontitis-derived PDLSC. | Increase of miR-17 expression in PDLSC might improve bone regeneration under inflammatory conditions. | 44 |
| miR-17 | TCF3        | ↑ in differentiation; ↑ by inhibitory concentrations of Wnt3a | Context-dependent regulation of miR-17 by Wnt3a. Inhibitory effect on osteogenic differentiation through TCF3 targeting. | Potential target to regulate Wnt canonical pathway activation during differentiation. | 45 |
| miR-17 | HDAC9       | Inhibitory loop between HDAC9 and miR-17 under inflammatory conditions. | Context-dependent role. Inhibits osteogenesis of healthy PDLSCs, promotes differentiation under inflammatory conditions. | Potential epigenetic control of periodontitis via pharmacological or direct targeting of HDAC9. | 46 |
| miR-21 | ACVR2B      | ↑ by stretch | Mechanically activated miR-21 promotes osteogenic differentiation by inhibition of ACVR2B. | Targeting ACVR2B-mediated signaling via miR-21 or mechanical stimulation as a strategy to improve bone regeneration. | 47 |
| miR-21 | SMAD5       | ↑ in osteogenic differentiation | miR-21 inhibits osteogenic differentiation of PDLSC by inhibiting the Smad5-Runx2 axis. | Targeting of miR-21 as a potential therapeutic tool for stimulating bone formation. | 48 |
| miR-21 | SPRY1       | ↑ by TNF-κ treatment | miR-21 promotes osteogenic differentiation of PDLSC by targeting SPRY1, negative regulator of ERK-MAPK and FGF pathways. | miR-21 as a possible therapeutic target in periodontitis and other inflammatory diseases. | 49 |
| miR-22 | HDAC6       | ↑ in osteogenic differentiation | miR-22 promotes osteogenic differentiation by targeting HDAC6, which is a co-repressor of RUNX2. | Promotion of osteogenesis by targeting miR-22/HDAC6 axis | 50 |
| miR-23a | BMPR1B     | ↑ in periodontitis-derived PDLSC | miR-23a inhibits osteogenic differentiation by targeting BMPR1B, leading to inhibition of Smad 1/5/9 phosphorylation. Periodontitis treatment decreases miR-23a expression. | miR-23a as a potential biomarker and target for the treatment of periodontitis. | 19 |
| miR-23a | SMAD5      | ↑ in osteogenic differentiation | miR-24-3p inhibits osteogenic differentiation by targeting SMAD5. | miR-24-3p as a potential target for periodontal disease treatment. | 51 |
| miR-132 | GDF5       | ↑ in osteogenic differentiation | miR-132 inhibits osteogenic differentiation of PDLSCs by activation of NF-κB through GDF5 targeting. | miR-132 as a potential target for periodontal disease treatment. | 52 |
| miR-138 | BGLAP      | ↑ in IL-6, TNF-α, and LPS-induced inflammation | miRNA-138 inhibits osteogenic differentiation by targeting osteocalcin gene expression directly and, probably, RUNX2. | miR-138 inhibitor as a potential therapeutic agent for the prevention of the bone loss associated with advanced periodontal disease | 53 |
| miR-24 | CTNNB1      | ↑ in osteogenic differentiation | miR-214 inhibits β-catenin gene expression and inhibits osteogenic differentiation. | Possible target to control Wnt canonical pathway and cell differentiation | 54 |
| miR-214 | RUNX2      | ↑ in osteogenic differentiation | miR-218 may inhibit osteogenic differentiation by targeting RUNX2. | Possible target to control cell differentiation. | 55 |
| miR-218 | TOB2       | ↑ in osteogenic differentiation | miR-543 promotes osteogenesis of PDLSCs by targeting TOB2, a cell cycle regulator. | Promotion of miR-543 expression as a potential approach for the treatment of periodontal bone loss. | 56 |
| miR-1305 | RUNX2     | ↑ after nicotine exposure | miR-1305 inhibits proliferation, migration, and osteogenic differentiation by inhibiting RUNX2 expression. | Control of miR-1305/RUNX2 pathway as a therapeutic strategy for the treatment of periodontal diseases. | 57 |
response to different stimuli, and the importance of addressing an appropriate research question when investigating mechanisms of differentiation at the molecular level.

In addition to those reported in Table 2, further studies identified miRNAs that are likely involved in the regulation of differentiation. Hao et al.\(^{58}\) identified by microarray analysis a miRNA profile for human PDLSCs induced to osteogenic differentiation. A total of 116 miRNAs were found to be differentially regulated, and the expression of 6 of them was validated by qPCR: miR-654-3p, miR-4288, and miR-34c-5p were confirmed as upregulated, while miR-218-5p, miR-663a, and miR-874-3p were downregulated during osteogenesis. Another paper described a miRNA signature for PDLSCs subjected to tension (10% equibiaxial strain at 1.0 Hz for 12 h).\(^{59}\) The authors identified 53 differentially regulated miRNA between normal and stretched PDLSCs, and validated the expression of miR-1246, miR-5096, miR-638, miR-663, miR-21, miR-4492, miR-4734 as upregulated and of miR-3195, miR-4281, miR-3178 as downregulated.

In accordance with the study by Chen reported in Table 2,\(^{57}\) miR-1305 together with miR-18b were found to be upregulated both after nicotine exposure \textit{in vitro}\(^{60}\) and in PDLSCs isolated from smokers.\(^{61}\) These miRNAs are associated with reduced proliferation, migration, and osteogenic differentiation of PDLSCs, therefore contributing to the pathogenesis of periodontal disease and affecting the regenerative potential of PDLSCs in smokers.

miR-210 was found to be upregulated in human PDLSCs by the presence of hydroxyapatite granules (Endobon\(^{®}\) Xenograft).\(^{62}\) The authors suggested a possible association between miR-210 and VEGF expression/secretion, though the target has not been clearly identified.

Finally, Zhou et al.\(^{14}\) showed that ibandronate, a nitrogen containing bisphosphonate, might promote proliferation and osteogenic differentiation of human PDLSCs and regulate the expression of osteogenesis-related miRNAs, such as miR-130a, miR-18a, mir-125b, and members of the miR-133 and miR-200 family.

**Expression and Role of lncRNA in Osteogenic Differentiation of Human PDLSCs**

lncRNAs represent a heterogeneous class of non-protein-coding transcripts, which are more than 200 nucleotides in length and can be several kilobases long. lncRNAs have limited or no encoding capability due to the lack of an extended open reading frame,\(^{63}\) but they are capable of regulating transcriptional complexes and chromatin structure.\(^{64,65}\) Therefore, lncRNAs regulate the expression of genes and miRNA through a number of different mechanisms\(^{66}\) (Figure 3) and are now recognized to play an important role in the regulation of key cellular processes, such as cell growth, differentiation, and apoptosis.\(^{67-69}\)
have an important role in determining cell fate, and some key members of this family have been recognized to regulate osteogenesis. 70

During the osteogenic differentiation of human PDLSCs, several lncRNAs are differentially expressed. 71-73 For instance, downregulation of anti-differentiation non-coding RNA (ANCOR) inhibits osteogenic differentiation of human PDLSCs. 74 Moreover, lnc Taurine upregulated gene 1 (TUG1) promotes the expression of runt-related transcription factor 2 (RUNX2), osteocalcin (OCN), and osteogenic-related gene marker alkaline phosphatase (ALP) level in human PDLSCs. 75

In the paper by Qu et al, 76 994 upregulated and 1177 downregulated lncRNAs were identified by microarray analysis, and those fell into different types, including antisense, enhancer-like lncRNA, and long intergenic non-coding RNA (lincRNA). Interestingly, 5 different transcript isoforms of lncRNA MEG3 were found to be significantly upregulated, supporting a positive role of this lncRNA in osteogenic differentiation in accordance with other studies in PDLSCs 77 and in bone marrow MSCs derived from multiple myeloma patients. 26

Another study used RNA sequencing to identify 960 differentially expressed lncRNA in PDLSCs osteogenesis. Together with differentially expressed circular RNAs (which will be discussed more in details within the next section), many of these transcripts are predicted to bind several miRNAs, leading to the regulation of hundreds of messenger RNAs involved in key signaling pathways of osteoblast differentiation and stem cell pluripotency. 72 LncRNA differential expression was also observed during osteogenic differentiation in a TNF-α induced inflammatory environment. 73

The identification of lncRNA which are differentially expressed during differentiation is of pivotal importance to identify targets that should be studied more in details. However, the validation of expression, and the identification of precise molecular mechanisms is required in order to better understand and exploit the function of ncRNAs. Table 3 summarizes the role of specific lncRNA, with identified targets and specific functions, in osteogenic differentiation of human PDLSCs.

LncRNA-ANCOR was reported to inhibit osteogenic differentiation of human PDLSCs by regulating Notch and Wnt pathways. 74,78 In the case of Notch, this lncRNA acts as a miRNA sponge to relieve the inhibition on NOTCH2 expression by miR-758. In short, lncRNA-ANCOR increases NOTCH2 expression, leading to an inhibition of the osteogenic program. On the other hand, the down-regulation of lncRNA-ANCOR leads to the activation of Wnt pathway, an important regulator of osteoblast differentiation.

Other lncRNA have been reported to have a promoting role in osteogenic differentiation of human PDLSCs, such as MEG3, 77 TUG1, 75 lncRNA-TWIST1 79 and lncRNA-POIR. 21 The mechanisms of action are different, with MEG3 and lncRNA-POIR acting as miRNA sponges, TUG1 promoting Lin28a expression, and lncRNA-TWIST1 inhibiting the expression of its protein-coding counterpart. Nevertheless, they all contribute to the regulation of osteogenic differentiation by regulating important pathways such as Wnt or insulin signaling.

Expression and Role of circRNA in Osteogenic Differentiation of Human PDLSCs

CircRNAs are RNA molecules with a wide size range, with ends covalently closed to form the peculiar circular structures. 33 CircRNAs have several regulatory functions, including that of miRNA and RNA binding-protein sponges, 34 which hinder their accessibility to target molecules (Figure 4). The role of circRNAs in regulating key cellular processes during stem cell differentiation has recently started to be acknowledged. Differential expression profiles of circRNAs in osteogenic differentiation of bone marrow 22,80 or adipose 81 derived MSCs was identified, and the function of some of them is being studied in more details. 82,83

Only a few studies identified circRNA expression profiles or functions in osteogenic differentiation of human PDLSCs. Zheng et al 84 analyzed the expression profile of circRNA (together with mRNA and miRNA) during osteogenic differentiation of human PDLSCs, resulting in the construction of a circRNA-miRNA-mRNA network. In the above-mentioned paper by Gu et al, 72 the authors analyzed both lncRNA and circRNA as possible competing endogenous RNAs. In particular, circRNAs derived from BANP and ITCH genes are suggested to regulate MAPK signaling pathway and osteogenic differentiation of PDLSCs via interaction with miR-34a and miR-146a.

Circular CDR1as, also known as cIRS-7, is one of the most studied circRNA, and it possesses several binding sites for different miRNAs, including at least 70 repeated miR-7 seed sequences. 85 Recently, CDR1as was found to be significantly upregulated during osteogenic differentiation of human PDLSCs and this was associated to miR-7 downregulation, 86 highlighting the miRNA-sponging mechanism in this system as also reported in other contexts. One of the targets of miR-7 appears to be GDF5, with downstream Smad1/5/8 and MAPK activities regulated. Interestingly, CDR1as also appears to be upregulated in LPS-induced PDLSCs 87 with a role in the regulation of ERK pathway to rescue LPS-induced inhibition of proliferation.

Overall, circRNA holds promise for the development of new therapeutic strategies to improve osteogenic differentiation of PDLSCs and to control response to inflammation. Definitely, more studies are required in this new field of molecular biology and gene expression regulation.
| lncRNA               | Target | IncRNA regulation in human PDLSCs | Role of IncRNA in osteogenic differentiation of human PDLSC | Translational potential                                                                 | Ref |
|---------------------|--------|-----------------------------------|-----------------------------------------------------------|----------------------------------------------------------------------------------------|-----|
| DANCR (ANCR)        | miR-758| ↓ in osteogenic differentiation    | IncRNA-ANCR inhibits osteogenic differentiation by sponging miR-758, ultimately affecting NOTCH2 expression. | LncRNA-ANCR/miR-758/Notch2 as a novel pathway for regulating osteogenic differentiation of PDLSCs | 78  |
| MEG3                | miR-27a-3p | ↓ in periodontitis-derived PDLSCs compared to healthy ones. | LncRNA-MEG3 promotes osteogenic differentiation by enhancing expression of IGF1 through miR-27a-3p sponging. | Regulation of MEG3 or miR-27a-3p/IGF1 axis as a potential therapeutic approach in periodontitis | 77  |
| TUG1                | Lin28A  | ↑ in osteogenic differentiation    | TUG1 promotes osteogenic differentiation of PDLSCs through Lin28A upregulation and binding. Positive cross regulation of TUG1 and Lin28A during PDLSCs osteogenic differentiation. | TUG1 as a potential tissue engineering approach to guide bone regeneration. | 75  |
| IncRNA-TWIST1       | TWIST1  | ↑ in osteogenic differentiation    | LncRNA-TWIST1 promotes osteogenic differentiation by inhibiting TWIST1 expression. TWIST1 downregulation increases β-catenin and RUNX2 expression. | Potential for IncRNA-TWIST1 as a therapeutic strategy in dental regenerative medicine. | 79  |
| IncRNA-POIR (ENST000004446358) | miR-182 | ↓ in osteogenic differentiation in periodontitis-derived PDLSCs compared to healthy ones. | IncRNA-POIR positively regulated osteogenic differentiation of healthy and periodontitis-derived PDLSCs both in vitro and in vivo, by sponging miR-182 and ultimately upregulating FOXO1. IncRNA-POIR and miR-182 suppress each other and form a network to regulate FoxO1, with inflammation increasing miR-182 expression. | IncRNA-POIR and miR-182 are identified as possible targets for periodontitis treatment. | 21  |
Clinical Utility and Current Limitations in ncRNA Research and Use

As of January 2020, around 300 actively recruiting clinical studies that include the study or use of miRNA and/or other ncRNAs are registered on ClinicalTrial.gov. This number indicates that there is a large interest in the introduction of ncRNA into the clinical practice; however, further considerations are needed. First of all, a large proportion of the clinical studies fall within the categories of cancer, cardiovascular, respiratory, and gastrointestinal diseases, and no study is directly related to any craniomaxillofacial condition. Also, most of the studies seem to focus on the use of miRNA (and in a much smaller proportion of lncRNA and circRNA) for diagnosis, prognostic evaluation, or drug follow up. While this represents an essentially important tool for precision medicine and for a better understanding of underlying pathophysiological mechanisms, the potential of ncRNA as therapeutic agents seem not to be extensively exploited to date outside basic research and preclinical studies. The development of miRNA-based therapeutics is nonetheless advancing, and recent reviews sum up the state of art about artificial miRNAs challenges and opportunities, plus drugs and companies currently working with miRNA for clinical practice.

The reason for this might rely in the relatively recent discovery of the different ncRNA types. The mechanism of RNA interference by small interfering (si)RNAs was first identified in 1993 in the nematode C. elegans, and only in the early 2000s the importance of this mechanism of gene expression regulation started to be acknowledged in other organisms and in humans. The same principles may apply to lncRNA and circRNA, which are also more complex than miRNA and with a plethora of possible mechanisms of action and functions that need a more extensive study.

LncRNAs have important roles in the pathogenesis of periodontal disease via regulation of many signaling pathways, some of them being revealed through high-

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Figure 4. The schematic summarizes some of the main mechanisms of action of circRNA to regulate gene expression, both pre- and post-transcriptionally. (A) Mainly studied is the miRNA/protein sponging function. The sequence derived from the pre-mRNA retains multiple miRNA and protein binding sites. Therefore, the presence of the circRNA acts as a decoy and hinders the ability of miRNA/proteins to reach their target sites on the mRNA. (B) Similar to lncRNA, circRNA can help recruiting and assembling protein together, that could be used for example to facilitate transcription factors complex interaction with the target gene promoter. (C) It is also believed that circRNA formation can compete with the linear mRNA for splicing, therefore regulating the cognate mRNA expression. Figure created with BioRender.com.
throughput next generation sequencing methods.\textsuperscript{92} Further evaluation of functional links between lncRNAs and their role in mediating osteogenic differentiation of PDLSCs may facilitate the determination of complex molecular mechanisms to better treat periodontal diseases. These comprehensive protocols would also aid in designing personalized procedures in this aspect.\textsuperscript{93} Besides, lncRNAs may be considered as therapeutic targets in periodontal disease. Forced siRNA-mediated downregulation of some lncRNAs or their overexpression could influence the pathologic process of periodontal disease, yet these protocols have limited clinical applications so far because of the delivery methods and safety concerns.\textsuperscript{93}

circRNA were discovered recently and more in-depth basic research still needs to be conducted in order to better understand how to exploit and tune their properties. circRNAs represent a new area for investigation in the field of gene expression regulation, and more rigorous research is needed to clarify their role in osteogenic differentiation of PDLSCs and in periodontitis. Also, once circRNA functions and role will be clearer, they can be engineered, or their expression manipulated,\textsuperscript{94-100} making circRNA as promising new tools for biotechnological approaches in CMF bone regeneration and tissue engineering. Using engineered circRNAs as therapeutic agents also poses a further challenge, as circular RNA structures can be detected by the immune system as they can be recognized as potential threats, such as of viral origin. Thus, the design of therapeutic agents based on circRNAs should be also done carefully to address this problem. Some studies focused specifically on this aspect,\textsuperscript{101-104} realizing that the presence of the m6A modification\textsuperscript{105} and of human introns\textsuperscript{104} might be important to suppress innate immunity.

Altogether, it is foreseeable that the advances in basic research within the field of ncRNA, unravelling new and still not completely clear molecular mechanisms, in the future will bring to better patient care. A closer connection between the clinicians and researchers with different backgrounds (e.g., cell and molecular biologists, chemists and chemical engineers, bioinformaticians, computational scientists), resulting in an interdisciplinary approach, will benefit and accelerate the translation of basic research results to clinical practice.

Conclusions

This review has provided insights into the role of different ncRNA types and their modulatory network in regulating osteogenic differentiation of human PDLSCs. Owing to their osteogenic differentiation ability, PDLSCs demonstrate efficient potential in the clinical application of periodontium repair and regeneration. A better understanding of the molecular mechanisms involved in bone regeneration or in the pathogenesis of periodontal bone loss will benefit future clinical applications. Indeed, this might help in the identification of novel molecular actors which can be potentially used as biomarkers for disease detection and follow up procedures, or as therapeutic targets/agents to treat certain bone-related oral diseases.

Moreover, since PDLSCs are often considered as potential sources of cells for bone tissue engineering strategies, it is necessary to understand how the microenvironment might affect them at a molecular level in order to design the best strategies of intervention.

Authors’ note

E.D.B. is Deputy Editor for the Basic Science & Molecular Biology section of CMTRO.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This investigation was supported by AO Foundation and AO CMF.

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