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REVIEW ARTICLE

Enhancing Osteogenesis in Dental Implants: ZNF710-AS1 and miR-146a-5p in Smad Signaling Pathway Activation

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ABSTRACT

Investigations have shown that understanding the regulatory mechanisms of the osteogenic differentiation of periodontal ligament stem cells (PDLSCs) can significantly enhance the regenerative capacity of periodontal tissues and mitigate periodontal disorders. Improving osteogenesis in dental implants is complex, requiring better bone regeneration and integration with the implant surface. ZNF710-AS1 is identified as a key regulator of osteogenic differentiation via the BMP6/Smad pathway, with the overexpression of miR-146a-5p and miR-146b-5p inhibiting ZNF710-AS1's osteogenic effects. This study explores the role of the long noncoding RNA ZNF710-AS1 in PDLSC osteogenic differentiation and clarifies its molecular mechanisms. Results indicate that ZNF710-AS1 expression positively influences osteogenic differentiation, while miR-146a-5p has a suppressive effect. ZNF710-AS1 serves as a competing endogenous RNA (ceRNA) for miR-146a-5p, increasing BMP6 levels and activating the Smad signaling pathway. The study also highlights the role of miR-146a-5p in osteogenesis and inflammation in peri-implant tissues. Findings suggest miR-146a-5p promotes osteogenic differentiation and could be a potential biomarker for periodontal conditions. In summary, this research provided key insights into the molecular mechanisms of osteogenesis and advocates for targeting ZNF710-AS1 and miR-146a-5p to improve dental implant integration and regenerative strategies for periodontal tissues.

Keywords: Osteogenic differentiation, ZNF710-AS1, miR-146a-5p, Regulation

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Introduction

Dental implant complications represent a critical issue within dental surgery, influencing not only the efficacy of the procedure but also the overall satisfaction of patients. Such complications may stem from many factors, encompassing technical, biological, and procedural challenges. A comprehensive understanding of these complications is essential for enhancing patient outcomes and reducing the risks linked to dental implants (1,2).

Peri-implant osteogenesis plays a crucial role in ensuring the stability and functionality of dental implants. This process is governed by the intricate interplay among osteoblasts, osteoclasts, and osteocytes. Small molecules influence osteogenic processes by initiating specific signaling pathways within cells. The activation of these pathways subsequently leads to the regulation and expression of particular target genes (3). Consequently, understanding the mechanisms of each pathway is crucial for comprehending the role of small molecules in promoting osteogenic differentiation. BMP/Smad signaling is recognized as a significant pathway in osteogenesis. This signaling pathway is characterized by its dual role, promoting both osteogenic and adipogenic processes (4).

Accumulating research has shed light on the significant functions of long non-coding RNAs (lncRNAs) in osteogenesis. An increasing array of lncRNAs has been identified as being differentially expressed during osteogenic processes. These lncRNAs have been further validated for their roles in regulating osteogenic markers and critical pathways involved in osteogenic differentiation, including the Wnt/β-catenin signaling pathway(5). The mechanisms by which competing endogenous RNAs (ceRNAs) operate during osteogenesis have been elucidated, revealing a notable interaction between lncRNAs and microRNAs (miRNAs), which has been extensively documented in the context of osteogenesis (6).

Emerging research increasingly suggests that non-coding RNAs (ncRNAs) are integral to the onset and progression of bone diseases, presenting opportunities for their utilization as biomarkers and therapeutic targets in the diagnosis, prognosis, and treatment of these conditions(6). Bone morphogenetic proteins (BMPs) belong to the transforming growth factor (TGF)-β superfamily and are crucial for bone regeneration processes. Osteogenic implants incorporating BMP-2 and BMP-7 have been effectively employed in the managing cases of long bone nonunion, spinal fusion, and acute fractures. Furthermore, BMPs have been identified as prognostic indicators for various bone diseases (6,7).

The small mother against decapentaplegics (Smads) functions as signal transduction molecules downstream of BMPs, with the BMP/Smad signaling pathway serving as a fundamental mechanism for the regulation of osteogenic differentiation(8). NcRNAs engage with the BMP/Smad signaling pathway, either directly or indirectly, facilitating the differentiation of stem cells into osteoblasts or promoting bone formation. The interplay between ncRNAs and the BMP/Smad pathway plays a significant role in modulating stem cell proliferation, differentiation, and angiogenesis during bone regeneration. Consequently, ncRNAs that interact with BMP/Smad pathway components represent promising targets for advancing bone regeneration and addressing bone disease diagnosis, prevention, and treatment(8).

Advanced high-throughput technologies, including RNA sequencing (RNA-Seq) and microarray profiling, have been utilized to explore the expression patterns of lncRNAs during osteogenic differentiation. These methods have effectively characterized a range of lncRNAs associated with osteogenesis(9,10). Identifying functional lncRNAs in osteogenesis has primarily concentrated on the different types of mesenchymal stem cells (MSCs) derived from various origins, such as embryonic tissue and bone marrow. Additionally, lncRNAs function as scaffolds or guides that influence the activity of essential regulators (11).

The Smad protein family serves as a crucial intermediary that conveys the signal initiated by the interaction between TGF-β and its receptor, facilitating the transfer of this signal from the cytoplasm to the nucleus. This process is essential for effective signal transduction and the regulation of transcription for downstream target genes (8). Recently, ZNF710-AS1 has been found to interact with specific miRNAs during PDLSC differentiation and it was identified as a key regulator of osteogenic differentiation through the BMP6/Smad pathway (11). ZNF710-AS1 expression was significantly upregulated during osteogenic differentiation and time-dependent expression changes were observed in PDLSCs under differentiation conditions. This review consolidates

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existing evidence on the differential expression and molecular mechanisms of lncRNA ZNF710-AS1, shedding light on its role in osteogenesis.

Literature Search and Selection of Articles

An extensive review of the current literature on the role of ZNF710-AS1 and miR-146a-5p in Smad signaling pathway activation in dental implants osteogenesis was undertaken. The inclusion criteria encompassed articles written in English, available in full-text, comprehensive, and directly pertinent to the subject under investigation. An extensive search was carried out in the PubMed and Scopus databases in December 2023, utilizing keywords related to long non-coding RNAs, lncRNAs, small mother against decapentaplegics, Smad, dental implants, osteogenesis, ZNF710-AS1, microRNAs, miRNAs, and miR-146a-5p. Initially, 85 articles were identified based on their titles, abstracts, and publication dates.

After eliminating duplicate entries, 42 distinct articles were retained. These articles were thoroughly analyzed, and a subset of 5 articles relevant to the research question was selected. Subsequently, in March 2024, a supplementary search was conducted using Google Scholar, PubMed, and Scopus, identifying and including 3 additional articles directly related to the topic of interest.

Historical Context

Since 1952, the universities of Lund and Goteborg have conducted extensive research on osseointegration. This concept originated from detailed microscopic examinations of rabbit fibula bone marrow, performed using minimally invasive surgical techniques and analyzed at high magnification with a specialized intravital microscope. In the early 1960s, investigations focused on bone marrow and joint tissue responses to various forms of injury, including mechanical, chemical, thermal, and rheologic damage (12).

The potential for osseointegration became apparent in many of these studies. Notably, Branemark, during his examination of microcirculation, noted that bone tissue could infiltrate narrow gaps in titanium, leading to an inseparable integration of titanium chambers within the bone structure (13). In the mid-1970s, Schroeder provided the first histological confirmation of osseointegration, demonstrating direct contact between bone and implant (14). Long non-coding RNAs (lncRNAs) have been recognized as crucial regulators in a variety of biological processes, including osseointegration, which refers to the integration of bone with implants. The exploration of these molecules has progressed significantly since the discovery of regulatory non-coding RNAs more than three decades ago (15).

Recent investigations, such as those conducted by Zheng (2020), have identified particular lncRNAs that facilitate bone formation in conjunction with implant materials. These discoveries imply that lncRNAs are vital in promoting osseointegration and may also be associated with conditions such as periodontitis, thereby underscoring their importance in bone health and integration methodologies (16).

The Cellular and Molecular Physiology of Osseointegration

Dental implants are extensively used in dental medicine as a restorative intervention to address complete or partial edentulism. The efficacy of oral implantation is contingent upon the successful synchronization of tissue healing and regenerative processes at the interface between the implant and bone, ultimately resulting in a dental implant that achieves “osseointegration” with the alveolar bone (17,18). This phenomenon transpires through a sophisticated sequence of biological processes that establish a direct structural and functional association between the surface of a load-bearing implant and living osseous tissue.

Nonetheless, this review will concentrate on the cellular mechanisms and signaling pathways pertinent to the osseointegration of titanium dental implants (17,19,20). Osseointegration commences as a genuine foreign body reaction (FBR) to biomaterials. Its primary objective is to encapsulate the foreign implant material through a multifaceted healing mechanism. The initiation of cellular events is characterized by an immune-modulated inflammatory response, followed by bone development and remodeling.

The peri-implant immune cells are pivotal in shaping the local microenvironment, as effective osteoimmunomodulation is crucial for achieving optimal osseointegration(18). Additionally, angiogenesis constitutes a significant factor in the success of osseointegration. Peri-implant osteogenesis is vital for the stability and functionality of dental implants, governed by the dynamic interplay among osteoblasts, osteoclasts, and osteocytes. Ultimately, a balance or

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equilibrium of the foreign body reaction (FBE) is attained through successfully integrating the implant with the bone (19).

Periodontitis exerts a considerable influence on osseointegration due to its associated inflammatory processes, which may impede the healing and integration of dental implants. The immune and inflammatory responses triggered by microbial agents in periodontitis can modify the regenerative capacity of PDLSCs (21). These stem cells are crucial for tissue repair and regeneration, as they exhibit both regenerative and immunomodulatory properties. By elucidating the relationship between inflammation in periodontitis and the functionality of PDLSCs, it becomes possible to devise novel regenerative therapies aimed at enhancing healing and optimizing the immune microenvironment, thereby improving outcomes for periodontal tissue repair (22).

Osseointegration represents a physiological phenomenon that occurs during the bonding of bone to an implant, paralleling the mechanisms of primary bone healing. Initially, upon the insertion of the implant into the bone, a layer of water is rapidly established around it within nanoseconds, which aids in the absorption of proteins and other critical biomolecules. Subsequently, over a timeframe ranging from 30 seconds to several hours, intercellular matrix proteins, originating from both interstitial fluid and blood, as well as from subsequent cellular activities, begin to coat the surface of the implant (14). This coating is characterized by a structure, composition, and orientation influenced by the surface type present. Additional modifications are made possible by extracellular matrix (ECM) proteins, cytoskeletal proteins, cell surface-binding proteins, the specific binding topography, chemical properties, and ion release from the implant (23).

The ECM proteins are crucial in conveying interpretable signals to the cells and their cohesive structures, which influence cellular shape, mobility, polarity, gene expression, survival, and proliferation. This signaling is mediated by various proteins, including collagen I, fibronectin, osteopontin, osteonectin, osteocalcin, bone sialoprotein, and specific plasma proteins such as α2HS glycoprotein, which serve as interfaces for cell adhesion and as communicators for interactions between cells and proteins (24). Positive osseointegration is influenced by a complex interplay of several confounding factors, including the biocompatibility of the implant material, the macro- and microscopic surface topography of the implant, the design characteristics of the implant, the morphology and quality of the bone at the implantation site, the surgical techniques utilized, the stability of both local and systemic health during the healing process, as well as the loading conditions and protocols implemented.

Clinicians face the significant challenge of managing all these factors concurrently to achieve successful osseointegration (25). Periodontitis is a plaque-induced inflammatory condition prevalent in the oral cavity characterized by the gradual deterioration of periodontal structures. It ranks among the foremost contributors to tooth loss. While traditional therapeutic interventions can mitigate active periodontal inflammation, they fail to region the compromised periodontal apparatus comprehensively (24).

The PDL constitutes a delicate layer of fibrous connective tissue between the alveolar bone and cementum, and it is integral to the development, function, and regeneration of the tooth-supporting framework. Subsequent research has confirmed that PDL cells form a heterogeneous population comprising fibroblastic and osteoblastic mesenchymal lineages, which include cells at various stages of differentiation and lineage specification (24).

PDL stem cells (PDLSCs) were first isolated in 2004 and demonstrated to possess self-renewal properties and multipotent differentiation abilities. Many in vitro studies have indicated that PDLSCs can be differentiated into diverse cell types, including adipocytes, osteoblasts, chondrocytes, neurons, and hepatocytes(26). A comparative analysis between PDL cells and PDLSCs highlighted their similarities in terms of high proliferative capacity, multipotent differentiation potential, expression of mesenchymal surface markers, and ability to regenerate periodontal tissues in vivo. This investigation also illustrated the practicality and safety of employing autologous PDL cells for periodontal regenerative therapies in the individuals diagnosed with periodontitis (27).

MicroRNAs (miRNAs)

RNAs represent a diverse group of biomolecules categorized into two main types: protein-coding RNAs and non-protein-coding RNAs. The latter, which do not undergo translation into proteins, can be further

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divided into housekeeping RNAs—such as ribosomal RNAs, transfer RNAs, and small nuclear RNAs—and regulatory RNAs(28,29). Regulatory RNAs are distinguished by length, encompassing short non-coding RNAs and lncRNAs. miRNAs, a subset of short non-coding RNAs, typically range from 20 to 23 nucleotides and act as post-transcriptional repressors by binding to mRNA, thereby silencing specific target genes(30,31).

The influence of microRNAs on the process of osteogenesis primarily occurs through the modulation of the Smad/BMP signaling pathways. Long non-coding RNAs can directly impact the expression of these pathways or the transcription factors associated with osteogenesis. Notable examples of interactions between lncRNAs and microRNAs in the context of osteogenesis include MALAT1/miR-30, MALAT1/miR-214, LEF1-AS1/miR-24-3p, MCF2L-AS1/miR-33a, miR-146a-5p, and KCNQ1OT1/miR-214(32).

MiR-146a-5p is critically involved in dental implants, mainly through its functions in osteogenic differentiation and the modulation of inflammatory responses. This microRNA has emerged as a pivotal factor in differentiating dental stem cells, essential for successfully integrating dental implants (11). Furthermore, miR-146a-5p has been associated with inflammatory mechanisms pertinent to the health of peri-implant tissues. The subsequent sections will provide a detailed examination of these functions (6).

MiR-146a-5p facilitates osteo/odontogenic differentiation in various dental stem cells, including dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAPs), and PDLSCs, by targeting TRAF6 and inhibiting NF-κB signaling, miR-146a-5p reduces the pro-inflammatory cytokine production, which may help maintain a favorable inflammatory microenvironment for bone regeneration and implant integration. In the case of periodontal ligament stem cells, the upregulation of miR-146a-5p during osteoblastic differentiation indicates its role in promoting mineralization and the expression of osteogenic genes (24).

In bone marrow mesenchymal stem cells, miR-146a-5p inhibits osteoblast differentiation, and its inhibition of Sirt1 may hinder this process in BMSCs. This consideration is crucial for assessing the role of miR-146a-5p in dental implant osseointegration, indicating a complex influence on bone formation that could affect dental implant integration (33). MiR-146a-5p exhibits anti-inflammatory effects, as evidenced in models of temporomandibular joint osteoarthritis, where it diminishes inflammatory secretions and improves cartilage integrity (33).

Due to its varying expression levels, miR-146a has been recognized as a potential biomarker in the context of periodontal and peri-implant diseases. However, its specific involvement in peri-implantitis is still not fully understood (24). Additionally, this microRNA can counteract the inhibitory effects of bacterial lipopolysaccharides on osteogenesis in periodontal ligament cells, indicating its potential utility in addressing inflammation-related challenges associated with dental implants (24).

Long non-coding RNAs (lncRNAs)

In recent years, lncRNAs have garnered significant interest, representing a substantial and varied class of RNA molecules exceeding 200 nucleotides in length. Initially regarded as mere "transcriptional noise" within the genome, it has become evident that these lncRNAs may serve essential regulatory functions across numerous biological processes (8).

Nevertheless, due to their low conservation, extensive alternative splicing, and specific expression patterns related to tissue and development, many lncRNAs remain uncharacterized and lack assigned functions. Typically, lncRNAs engage with DNA, RNA, and protein molecules, or combinations thereof, to modulate gene expression at both transcriptional and post-transcriptional levels (34).

The ongoing advancements in gene sequencing methodologies have rendered the integration of high-throughput sequencing with bioinformatics analysis an essential scientific approach for the extensive identification of lncRNAs associated with osteogenic differentiation (35). Numerous researchers have compared samples exhibiting osteogenic induction and those that do not, derived from various stem cell types, subsequently identifying differentially expressed lncRNAs(36).

Osteogenesis and adipogenesis represent fundamentally contrasting processes. Encouraging osteogenic differentiation and suppressing adipogenic differentiation is paramount for bone development. The downregulation of lncRNA ZFAS1 not only enhances the differentiation of osteoblasts but also counteracts the stimulatory influence on adipogenesis via a critical regulator such as miR-499(37).

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LncRNA ZNF710-AS1 in cooperation with miR-146a-5p on Osteogenesis

Research has demonstrated that lncRNAs play a significant role in various diseases characterized by disrupted cellular regulation, including autoimmune disorders, neurological diseases, cardiovascular issues, and cancers. Recently, there has been a growing interest in the function of lncRNAs in regulating PDL cells. Evidence suggests that lncRNAs exhibit differential expression in PDL cells compared to other cell types and that their expression varies during biological processes (37). Furthermore, specific lncRNAs, such as maternally expressed gene 3 (MEG3), anti-differentiation non-coding RNA (ANCR), and taurine-upregulated gene 1 (TUG1), have been identified as key regulators of the regenerative abilities of PDL cells in both standard and inflammatory environments (5,38).

LncRNAs have been identified to perform various functions, including serving as sponges for miRNAs, sponges for RNA-binding proteins (RBPs), and regulators of mRNAs. In 2011, Salmena et al. introduced ceRNAs, suggesting that lncRNAs can bind to miRNAs in competition with coding RNAs, facilitating interactive roles among these molecules. As research in this area advances, the significance of this mechanism is becoming increasingly recognized (39). Substantial evidence indicates that lncRNAs can function as ceRNAs during the osteogenic differentiation of PDLSCs. For instance, the inhibition of lncRNA KCNQ1OT1 has been shown to suppress both the proliferation and osteogenic differentiation of PDLSCs by targeting the elevated levels of miR-24-3p (39).

The previous investigation utilized bioinformatics tools to identify potential target genes for ZNF710-AS1, specifically hsa-miR-146a-5p, and hsa-miR-146b-5p. A prior study conducted differential expression analysis to pinpoint dysregulated differentially expressed genes (DEGs) in the osteodifferentiation processes of dental pulp stem cells (DPSCs) and bone marrow stem cells (BMSCs), revealing eight significantly downregulated DEGs, including miR-146a-5p and miR-146b-5p(11). MiRNAs bind specifically to the 3′-untranslated regions (UTRs) of target mRNAs, leading to either the degradation of the target mRNA or the inhibition of its translation. Additionally, our analysis of the GSE159507 dataset identified BMP6 as a common target gene for miR-146a-5p and miR-146b-5p, with their binding confirmed through dual luciferase reporter assays (11).

Numerous researchers have observed that the irregular expression of lncRNAs or the concurrent activity of multiple lncRNAs is significantly associated with human stem cells' biological behaviors and functions. Notably, the lncRNA MCM3AP-AS1 exhibited a substantial increase during the osteogenic differentiation of PDLSCs, with its expression levels showing a positive correlation with alkaline phosphatase (ALP) and Runx2. Furthermore, the lncRNA ZFAS1 was identified as a suppressor of osteogenic differentiation while promoting adipogenic differentiation (40). In the light of the aforementioned research findings, bioinformatics analysis was employed to determine the significantly upregulated long non-coding RNA ZNF710-AS1 during the osteogenic differentiation of PDLSCs. Following this, primary PDLSCs were effectively isolated and cultured. Cell models that either stably overexpressed or silenced ZNF710-AS1 were developed to examine the impact of ZNF710-AS1 on the proliferation and osteogenic differentiation of PDLSCs at both the gene and protein levels (41).

Previous research has established that the TGF-β/SMADs signaling pathway is crucial for the commitment and differentiation of osteoblast lineages, as well as for skeletal development and homeostasis. This pathway is initiated by the TGF-β receptor, a heterodimeric complex composed of type I and type II receptors located on the plasma membrane (42). The activation of this receptor results in the phosphorylation of the transcription factors SMAD2 and SMAD3, which then translocate to the nucleus to initiate gene transcription. Furthermore, specific non-coding RNAs (ncRNAs) have been identified as upstream regulators of the TGF-β/SMADs signaling pathway, including miR-34a-5p, linked to inflammatory responses in osteoblasts (43).

The other study by Zhang L et al. in 2022 showed that the miR-34a-5p/SMAD2 signaling axis promotes bone formation in models of infected bone nonunion and mitigates the inhibitory effects of lipopolysaccharide on osteoblasts. Studies have indicated that long non-coding RNAs can competitively bind to miRNAs, thereby indirectly influencing the expression of target genes through a sponge-like mechanism (44). LncRNA MALAT1 is a sponge for miR-34a-5p in cancer cells and enhances

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osteoblast activity in osteoporotic mice by modulating the miR-34c/SATB2 axis. Nevertheless, the mechanisms by which LncRNA MALAT1 influences osteoblast proliferation and differentiation in the context of infected bone defects, as well as the regulatory role of ECP on lncRNA MALAT1 in inflammatory bowel diseases, remain to be elucidated(45,46).

Hakki et al. conducted a study utilizing quantitative RT-PCR to assess the mRNA expression levels of type I collagen, bone sialoprotein, osteocalcin, osteopontin, and the osteoblast transcription factor Runx2 in PDLSCs subjected to treatment with BMP2, BMP6, and BMP7(47). Their findings indicated that the most significant induction was observed in the BMP6 treatment group. Previous research has established that BMP6 enhances the transcriptional activity of the Smad1/5/9 signaling pathway, facilitating the translocation of these proteins into the nucleus and subsequently promoting the osteogenic differentiation of MSCs (11,47).

In the published investigation, the levels of BMP6 and phosphorylated Smad1/5/9 proteins were found to be either upregulated or downregulated following the overexpression of ZNF710-AS1 or miR-146a-5p/miR-146b-5p. Furthermore, the overexpression of miR-146a-5p/miR-146b-5p negated the osteogenic differentiation enhancement induced by ZNF710-AS1 upregulation in PDLSCs (11). A reduction in calcium deposition, alkaline phosphatase activity, and the expression of osteogenic proteins evidenced this. ZNF710-AS1 plays a down-regulative role in the osteogenic differentiation of PDLSCs by activating the expression of the BMP6/Smad1/5/9 pathway, functioning as a ceRNA for miR-146a-5p and miR-146b-5p. Numerous studies have investigated the function of MALAT1 in the process of osteogenesis. Che et al. were the first to identify that MALAT1 influences the expression of OPG in hFOB1.19 bone cells, although the precise connection between MALAT1 and osteoblast differentiation remained ambiguous (48).

Later, Xiao et al. reported a significant increase in MALAT1 expression in calcified valves and hAVICs during osteogenesis. In this context, MALAT1 functioned as a competing endogenous RNA (ceRNA), enhancing Smad4 levels by sequestering miR-204. This mechanism led to upregulating osteoblast-specific markers, including ALP and OCN, thereby facilitating bone matrix formation in hAVICs (49). While the long non-coding RNA H19 is recognized as one of the most prevalent and evolutionarily conserved non-coding transcripts during mammalian development, significantly influencing processes such as proliferation, differentiation, and carcinogenesis(50). The initial suggestion of H19's notable upregulation during osteogenesis was made by Huang et al., and this was later validated by additional research involving osteoblasts and human bone marrow-derived stem cells. These discoveries have sparked considerable scholarly interest in the function of H19 within the context of osteogenesis (51) (Figure 1).

Highly expressed lncRNA-ZNF710-AS1 was found to competitively sponge miR146a-5p/miR-146b-5p, which was thought to enhance the ability of PDLSCs to differentiate into osteoblasts. Extensive experimental evidence has established that a comprehensive understanding of the regulatory mechanisms governing the osteogenic differentiation of PDLSCs can significantly enhance the regenerative capacity of periodontal tissues and mitigate periodontal diseases (41).

This study aimed to investigate the involvement of the lncRNA ZNF710-AS1 in the osteogenic differentiation of PDLSCs and to elucidate its underlying molecular mechanisms. Microarray datasets GSE159507 and GSE159508 were obtained from the Gene Expression Omnibus database, and differentially expressed genes were identified utilizing the R programming language (limma package) (11). The findings indicated that the expression levels of ZNF710-AS1 and BMP6 were elevated, while those of miR-146a-5p and miR-146b-5p were reduced during the osteogenic differentiation of PDLSCs.The overexpression of ZNF710-AS1 markedly enhanced the osteogenic differentiation potential of PDLSCs by increasing the expression of BMP6 and phosphorylated Smad family member 1/5/9 (p-Smad1/5/9) while also competitively sponging miR-146a-5p and miR-146b-5p, thereby functioning as a ceRNA(52,53).

Wang et al. demonstrated that the inhibition of lncRNA THAP9-AS1 could hinder the osteogenic differentiation of PDLSCs via the miR-652-3p/VEGFA signaling pathway. In the present study, an analysis of Gene Expression Omnibus (GEO) data (specifically the GSE159507 dataset) revealed an increase in the expression of ZNF710-AS1 during the osteogenic differentiation of PDLSCs. ZNF710-AS1,

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which spans approximately 7.6 kb, is situated on human chromosome 15q26.1 and is expressed across various human tissues (54). The findings indicated that during the osteogenic differentiation of PDLSCs, there was an upregulation in the expression of ZNF710-AS1 and bone morphogenetic protein 6 (BMP6), while the levels of miR-146a-5p and miR-146b-5p were downregulated. PDLSCs were effectively isolated and cultured in vitro (54). The overexpression of ZNF710-AS1 notably enhanced the osteogenic differentiation potential of PDLSCs by increasing the expression of BMP6 and phosphorylated Smad family members 1/5/9 (p-Smad1/5/9) while also competitively sponging miR-146a-5p and miR-146b-5p, thereby functioning as a ceRNA.

This research elucidated that ZNF710-AS1 facilitates the osteogenic differentiation of PDLSCs by upregulating BMP6 and Smad1/5/9 expression and serving as a ceRNA for miR-146a-5p and miR-146b-5p (54).

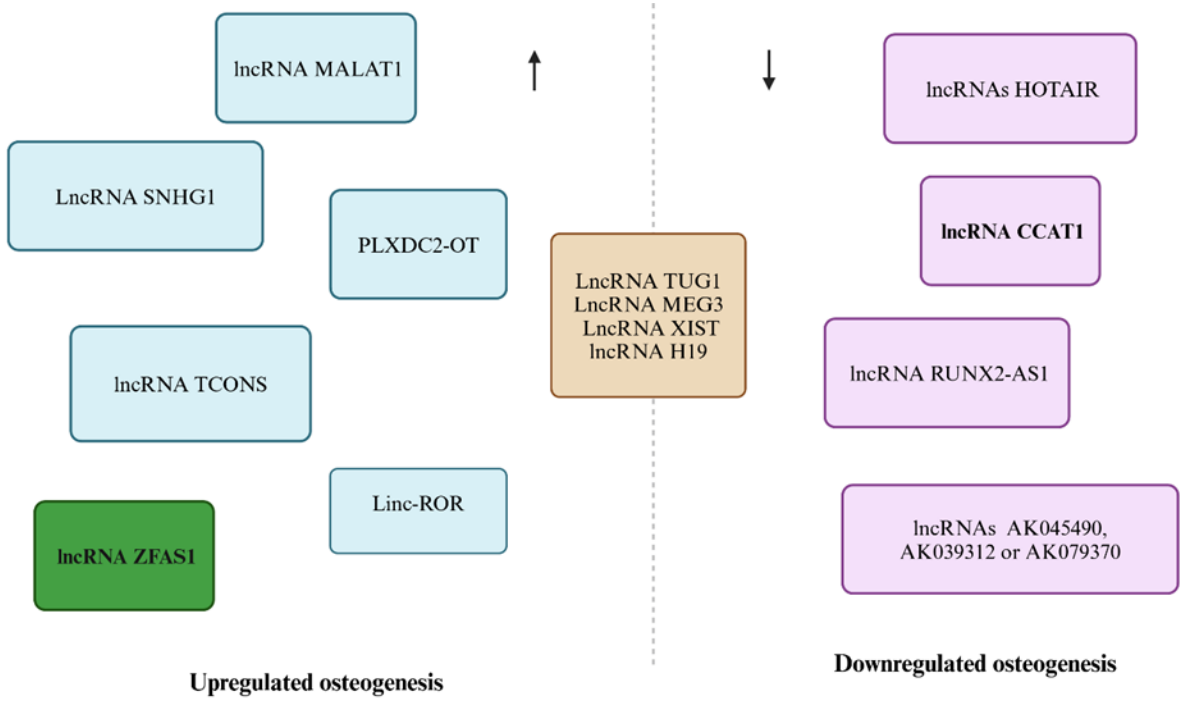

Figure 1. LncRNAs reveal upregulation, downregulation, and upregulation /downregulation role osteogenesis.

The interaction among the Smad signaling pathway, miR-146a-5p, and PDL cells constitutes a sophisticated regulatory framework that governs cellular differentiation and proliferation. miR-146a-5p is a microRNA that influences multiple signaling cascades by targeting specific genes, including those belonging to the Smad family, which play a pivotal role in the TGF-β signaling pathway(55). This relationship is significant in osteogenic differentiation, adipogenesis processes, cell proliferation, and apoptosis modulation. miR-146a-5p is a ceRNA sequestered by the lncRNA ZNF710-AS1 during the osteogenic differentiation of PDLSCs. This interaction promotes the activation of the BMP6/Smad1/5/9 signaling pathway, thereby facilitating osteogenic differentiation (55,56). The downregulation of miR-146a-5p correlates with increased BMP6 expression and phosphorylated Smad1/5/9, further supporting osteogenesis in PDLSCs (Figure 2).

In porcine intramuscular preadipocytes, miR-146a-5p inhibits adipogenesis by targeting Smad 4 and TRAF6, which reduces TGF-β and AKT/mTORC1 signaling pathways. This microRNA suppresses Smad 4, diminishing TGF-β signaling, a critical pathway for adipocyte differentiation (33). miR-146a-5p also targets Smad 3 and Smad4, thereby influencing cell proliferation and apoptosis. The inhibition of these Smad proteins by miR-146a-5p results in decreased cell proliferation and increased apoptosis, as demonstrated in studies involving porcine kidney cells and BeWo cells (54).

The modulation of these processes by miR-146a-5p holds considerable importance across various biological scenarios, particularly during the early

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stages of pregnancy in sows. The influence of miR-146a-5p extends to the attenuation of TGF-β signaling in the context of cellular senescence and its impact on the proliferation of acute promyelocytic leukemia cells via the TGF-β/Smad pathway (56).

These observations emphasize the potential of this microRNA as a therapeutic target in conditions characterized by dysregulated TGF-β signaling. Although miR-146a-5p is predominantly recognized for its regulatory functions in differentiation and proliferation through its engagement with the Smad pathway, its effects are contextually dependent, exhibiting variability across different cell types and environmental conditions. This variability highlights the intricate nature of miRNA-mediated regulation in cellular mechanisms (54).

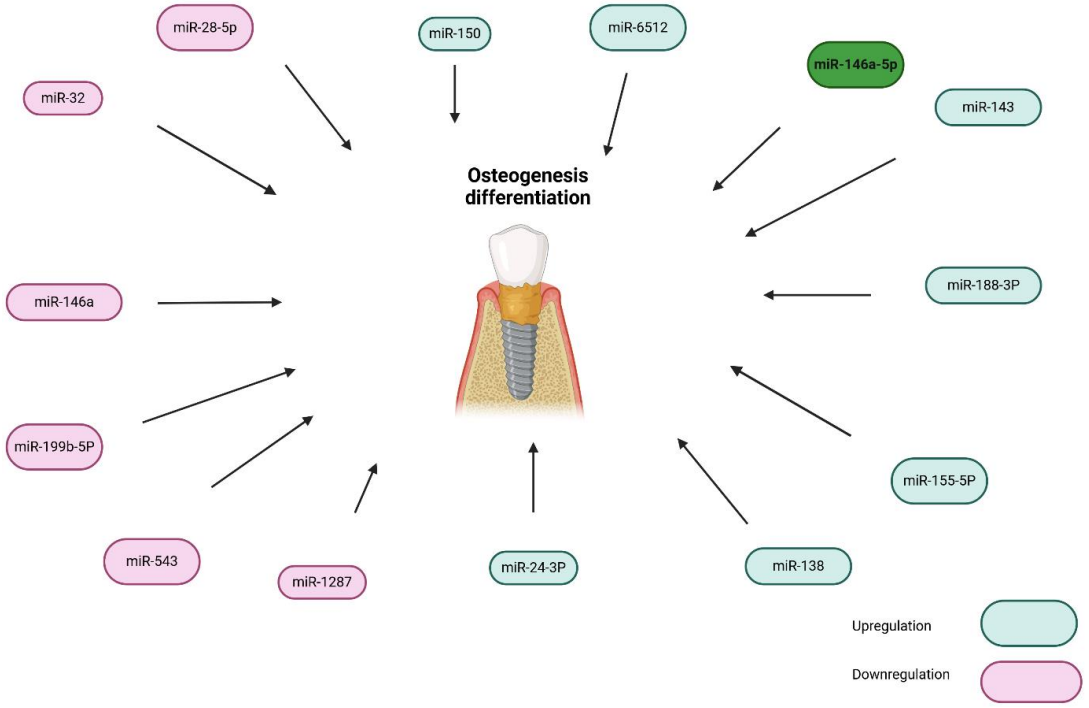

Figure 2. The miRNA cooperated in osteogenesis differentiation via downregulation (green boxes) or upregulation (pink boxes).

Li Q's study manifested that Linc01133 has been shown to promote osteogenic differentiation of human periodontal ligament stem cells by regulating the miR-30c/BGLAP axis, which acts as a competing endogenous RNA (57). Wang et al. identify lncRNA-POIR, which is associated with osteogenic differentiation in periodontal mesenchymal stem cells from periodontitis patients. LncRNA-POIR expression is significantly altered in hPDLSCs and pPDLSCs suggesting its potential role in enhancing bone formation relevant to dental implants, although it does not specifically address dental implants 57).

A prior investigation conducted a differential expression analysis to pinpoint dysregulated differentially expressed genes (DEGs) involved in the osteodifferentiation of DPSCs and BMSCs (58, 59). As a result, eight DEGs were identified as significantly downregulated, including miR-146a-5p and miR-146b-5p. The miRNAs bind specifically to the 3′-UTRs of target mRNAs, leading to either the degradation of the target mRNA or the inhibition of its translation. Additionally, within the GSE159507 dataset, the analysis identified BMP6 as a common target gene for both miR-146a-5p and miR-146b-5p, and this interaction was confirmed through dual luciferase reporter assays (29,35).

Additionally, overexpression of ZNF710-AS1 increased BMP6 levels and activated the Smad1/5/9 pathway, and overexpression of miR-146a-5p and miR-146b-5p inhibited the osteogenic effects of ZNF710-AS1. These miRNAs played a suppressive role in PDLSC osteogenic differentiation (58, 59).

Discussion

The findings of the current investigation indicate that ZNF710-AS1 plays a significant role in promoting the osteogenic differentiation of PDLSCs. The primary

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mechanism identified involves the action of a ceRNA for miR-146a-5p, which leads to an increase in BMP6 levels and subsequently activates the Smad signaling pathway. Based on the present results, ZNF710-AS1 acted as a competing endogenous RNA, regulating BMP6 via miR-146a-5p and miR-146b-5p and overexpression of ZNF710-AS1 increased BMP6 levels and activated the Smad1/5/9 pathway.

These findings enhance our comprehension of the molecular biological processes that govern the osteogenic differentiation of PDLSCs. Furthermore, these results offer a novel molecular target and a theoretical framework for regulating dental stem cells to foster oral tissue regeneration. The findings of this study indicate promising directions for future investigations, especially in examining the therapeutic potential of targeting ZNF710-AS1 and miR-146a-5p to facilitate dental implant integration and enhance regenerative results in periodontal treatments.

Avenues for future research

The findings suggest that manipulating the levels of ZNF710-AS1 could enhance the osteogenic differentiation of periodontal ligament stem cells (PDLSCs). This could lead to improved strategies for dental implant integration and periodontal tissue regeneration, making it a potential therapeutic target in clinical settings. Besides the study emphasizes the importance of the BMP/Smad signaling pathway in osteogenesis.

Future research could focus on elucidating the detailed molecular mechanisms by which ZNF710-AS1 and miR-146a-5p interact with this pathway, potentially leading to novel therapeutic interventions for bone regeneration. The promising results from this study warrant clinical trials to evaluate the efficacy of targeting ZNF710-AS1 and miR-146a-5p in enhancing dental implant success rates and improving outcomes in periodontal treatments. Such trials could validate the translational potential of these findings.

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