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# The mechanism and research progress of circRNA-microRNA in regulating osteogenesis

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***Abstract:*** *Jaw defect and alveolar bone absorption are the main oral problems in today's society. In recent years, studies have found that changes in the expression of different miRNAs can affect the osteogenic effects of stem cells. With the in-depth study of its upstream circRNA, it has been found that circRNA can pass it acts as a molecular sponge of microRNA to indirectly regulate osteogenesis, so how to select a suitable and stable circRNA has become the focus of attention. In this review, we have collected and sorted out circRNA and its functions, the application and process of mirRNA to regulate osteogenic differentiation, and a list of several miRNAs closely related to osteogenesis and relatively mature researches, aiming to find suitable circRNA upstream Therefore, it is expected to be used in the clinical repair of oral bone defects.*

***Keywords:*** *circRNA, microRNA, osteogenic differentiation jaw bone defect, tissue regeneration, bone marrow mesenchymal stem cells, molecular sponge*

## 1. Introduction

The absorption of jaw defect and alveolar bone is a common oral disease, which usually occurs in elderly patients, which not only limits the quality of life of patients, but also brings serious gastrointestinal burden, which has become the main oral problem in elderly patients in today's society. How the use of circRNA to regulate gene expression has become the main research direction of RNA field [1]. circRNA belongs to one of non-coding RNA that can influence cellular mechanisms on the molecular level, and studies have shown that they increase the translation and stability of such RNA by inhibiting microRNA,. In this review we discuss the function of circRNA including acting as bait for microRNA molecular sponge or RNA-binding protein to regulate gene expression or regulate protein translation, and the role of bone defects for various causes in the mouth in regulating new bone formation. Here, we briefly discuss their process and focus the discussion on the regulation of microRNA-induced ostegenesis through circRNA. Exploring the role of circRNA in promoting the occurrence and development of bone formation will help us to clarify the function of circRNA and facilitate the development of potential tools for early diagnosis and effective treatment of bone defects. Application of multidirectional differentiation potential of bone marrow mesenchymal stem cells and application of binding cell membrane

Bone marrow mesenchymal stem cells (BMSCs) is a class of multipotential stem cells derived from mammal bone marrow matrix, therefore have good multidirectional differentiation potential and self-renewal ability, which can be directed into various cell types such as osteosteocytes, chondrocytes, myocardial cells, nerve cells, fat cells, bringing new development prospects for tissue repair and regenerative medicine [2]. Research on BMSC has hitherto demonstrated that numerous transcription factors and extracellular or interstitial signaling pathways regulate fat formation and bone differentiation. It is found that specific circRNA in BMSCs plays a key role in maintaining the number, characteristics and differentiation direction of stem cells in the body, so circRNA shows attractive prospects as the regulatory gene of seed cell BMSCs in tissue engineering bone application.

Cell membrane technology was proposed by the Japanese scholar OKANO in 1993. [3] He is a

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membrane composed of a cell and extracellular matrix with a unique recomposite stent material that preserves the performance of the intercellular autosecreted signal factor, greatly improving the cell utilization and plasticity of the film. Yan Jun et al use liposome transdye antimiR-138 to BMSCs membranes to significantly enhance bone differentiation of cell membranes [4]. circrRNA can be integrated into the cell membrane to promote osteoblast differentiation.

## 2. Definition and function of the circRNA

Ring RNA (circulRNA, circRNA) as a different from previous non-coding RNA, is mainly in that it does not have the traditional 3 'end and 5' end hat of this RNA typical structure, but forms a unique single-chain loop RNA structure in the form of covalent bond [5]. Composition can be divided into exon source circRNA (ecircRNAs), intron source circRNA (ciRNAs), exon-intron source circRNA (EIciRNA), and intergene circRNAs (ic circRNAs). Cell localization studies show that ecircRNAs regulates the expression of miRNA target genes by "sponge" adsorption of tiny RNA (miRNA) located in the cytoplasm to play the role of other RNA within the antibody[6]; While ciRNAs is involved in regulating transcription and selective shear[4], located in the nucleus to regulates the transcription and splicing of the [8, 9]. It is found that the special single-chain loop structure of circRNA is attached to its biological properties different from other RNA s, such as relatively strong stability. circRNA has a wide, stable, diverse and other characteristics, but also has transcription regulation, protein translation, miRNA sponge and other functions. The transcriptional regulation function can participate in the regulation of transcription. The protein translation function of circRNA translation into protein is not only different from linear miRNA, but also the process may be more complex. CircRNA becomes the molecular sponge in MiRNA sponge and thus affects the function of miRNA, which is used to inhibit the translation of the target mRNA and enhance its stability, and finally plays the purpose of regulating miRNA.

## 3. Regulatory role of miRNA in osteoblast differentiation

Osteogenic differentiation of osteblasts is a process controlled by internal and external factors, in which miRNA plays an important role and is the key regulatory factor of stem cell bone formation. It endogenous regulates bone differentiation by directly positively or negatively targeting ostegenesis, and promote or inhibit bone differentiation accordingly. [10]

1) Bone marrow mesenchymal stem cells with silent microRNA-137-3p[11]The role of transplantation on the prevention and early treatment of femoral head necrosis) in the discovery of silencing miR-137-3p contributes to osteogenic differentiation of BMSC and also promotes angiogenesis of human umbilical venous endothelial cells (HUVEC) [12]. Protein imprint and double luciferase assays confirmed the direct targeting of miR-137-3p as Runx2 and CXCL12. They can play a key role in SONFH repair by promoting osteoblast differentiation and mobilizing EPC (endothelial progenitors) [13].

2) miR-185 negatively regulates bone production in vitro, in mice with miR-185 knockout, primary ostelasts and mesenchymal stem cells showed enhanced osteogenesis, dual fluorinase report gene detection identified bisaccharide chain proteoglycan (Bgn) as the direct target of miR-185 that inhibited Bgn expression during osteoblast differentiation. [14] The protein promotes bone formation through the BMP / Smad pathway. Primary osteoblasts knockout miR-185 mice were found to show enhanced proliferative capacity. Meanwhile, increased ALP activity in ALP was quantitatively measured after 7 days and 14 days of osteoblast induction in knockout miR-185 mice.The ALP staining results also showed a significant increase in ALP expression. Without the miR-185, the MSCs showed enhanced osteogenesis.

3) High bone formation expression of Wnt pathway for miR-335-5p segment in mouse gene [15] it has played an important role. Dickkopf-1 (DKK 1) can be an antagonist to slowing the wnt pathway. The reduction of its expression indicates the enhancement of the osteoblast differentiation ability. Thus, decutting of miR-335-5p precursor gene to form mature miRNA, can reduce the level of DKK1 and Bim factors, thus indirectly increasing the activity of wnt pathway and accelerating bone formation induction [16]

## 4. CircRNA regulates bone formation by sponging on microRNA

The sponge effect of the circRNA is to affect the downstream target gene, to inhibit the function of the miRNA, and finally to achieve the purpose of regulating the miRNA activity. [17] The first discovered

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circRNA with this function was CDR1as (ciRS-7), which was found that CDR1as is a miR-7 sponge with 63 conservative miR-7 action targets, combined with miR-7 and regulating its function. [21] This is also the earliest discovered sponge action of circRNA. With the development of sequencing and bioinformatics analysis technology, more and more CircRNA have been found and circbank database is increasingly rich, which provides a strong backing for the development of circRNA. With the increasing maturity of CircRNA sponge technology, in the past two years, mainly focusing on bone formation, providing a new treatment idea for the treatment of jaw defects.

1) Some scholars have done experiments on bone formation induction of maxillary sinus membrane stem cells. Under the premise of known regulatory relationship between miR-214-3p and maxillary sinus stem cell bone formation differentiation, because CircRNA can affect the function of miRNA, they predicted the target target genes related to circRNA_33287 by using online bioinformatics tools, with the highest relationship between miR-214-3p and the target circRNA [18] Then the relationship is further verified by detection methods such as high flux. In subsequent experiments it was also found that the content of associated osteogenic markers in maxillary sinus membrane stem cells when overexpressed or silent circRNA_33287 changed. Finally, the relationship between circRNA and miR-214-3p was confirmed by bone correlation index and histological analysis. This demonstrates that circRNA_33287 plays a role in regulating the osteoblast differentiation of maxillary sinus membrane stem cells by affecting miR-214-3p.

2) The emerging function of mm9_circ_009056 regulating bone morphogenetic protein 7 (BMP7) through miR-22-3p during bone formation, we found that calcitonin gene-related peptide (CGRP) had strong osteogenesis on MC3T3 cells, elevated mm9_circ_009056 expression in CGRP-induced cells, and found negative feedback expression of miR-22–3p. Silencing mm9_circ_009056 increases the expression of miR-22–3p and reduces osteogenesis-related gene and protein levels such as BMP7, RUNX2. It was found that protein levels like BMP7 and RUNX2 decreased after transfected with silenced mm9_circ_009056 and increased after transfected miR-22–3p inhibitors. In short, mm9_circ_009056 can act as a sponge of miR-22–3p, regulating cellular osteogenesis induced by CGRP [19].

3) Researchers in patients with bone disconnection found that transfected with treated overexpressed and depressed hsa_circRNA_0074834 in bone marrow mesenchymal stem cells, which promotes the expression of bone-related factors in BMSCs, but suppresses the expression of associated bone factors. Preast the most intimate miRNA-miR-942-5p. with hsa_circ_0074834 using bioinformatics methods The detection between bone correlation in subsequent experiments and histological asses confirmed the interaction between circRNA and miR-942-5p.Hsa_circ_0074834 acts as ceRNA to regulate the expression of ZEB1 and VE GF through microRNA-942-5p, which can significantly reduce the expression of ZEB1 and VE GF protein, while the inhibition of miR-942-5p function can promote the expression of ZEB1 and VE GF, which in turn promotes BMSC osteogenic differentiation and the repair of bone defects. [20].

*Table 1: List of Sponge Effects of circRNA*

| CircRNA Name | Target miRNA | Access, Target | Related diseases and their effects | reference documentation |
|---|---|---|---|---|
| Hsa_circRNA_33287 | miR-214-3p | Runx3 | Overexpression can promote the osteogenic differentiation of maxillary sinus membrane stem cells | [18] |
| Mm9_circ_009056 | miR-22-3p | CGRP | Overexpression can enhance osteogenesis | [19] |
| hsa_circ_0074834 | miR-942-5p | ZEB1&VEGF | Overexpression can promote the osteogenic differentiation of BMSCs and the repair of bone defects | [20] |
| hsa_circ_0026827 | miR-188-3p | Beclin1&RUNX1 | Overexpression can promote osteoblast differentiation of human dental pulp stem cells | [21] |
| CircRNA-SIPA1L1 | miR-204-5p | ALPL | Overexpression can promote bone differentiation of dental papilla stem cells | [22] |
| CircRNA-PRKD | miRNA-21 | mTORC1 | Regulation of osteogenic differentiation of periodontal ligament stem cells | [23] |
| circRNA -CDR1-AS | miR-7 | Smad1/5/8 & p38 mitogen-associated protein kinase | Promote the osteogenesis of periodontal ligament stem cells | [24] |
| mmu_circ_003795 | miR-1249-5p | COL15A1 | Regulates osteoblast differentiation and mineralization in MC3T3-E1 and MDPC23 | [25] |
| Circ_0024097 | miR-376b-3p | Wnt /β-catenin Hippo pathway | Promote osteogenic differentiation to reduce osteoporosis | [26] |
| circ-DAB1(has_circ_0113689) | miR-1270& miR-944 | NOTCH / RBPJ | Promote cell proliferation and osteogenic differentiation of BMSC | [27] |
| Circ_ORC2 | miR-19a | Inhibit downstream PTEN expression | Promote the growth and invasion of osteosarcoma cells | [28] |

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## 5. Summary

The ability of bone marrow mesenchymal stem cells to be induced to differentiate into osteoblasts [29] it can be applied in bone tissue engineering to promote bone formation and repair maxillofacial defects. Experiments have found that the bone formation differentiation of bone marrow mesenchymal stem cells is involved and regulated by many RNA [30]. The relationship of miRNA and induced bone formation has been studied by many scholars [31] The function of regulating bone formation was confirmed and based for the treatment of related diseases such as periodontitis [32]", Osteoporosis [33] Theoretical theory of gene therapy [34] However, the interaction and action mechanism between miRNA and its associated upstream circRNA are not very clear, requiring further research, we can predict the expression level of the circRNA, qRT-PCR gene to verify the osteogenesis by bone markers such as ALP, RUNX2, OSX, OPN, OC.

Numerous studies have shown that the vast majority of circRNA acting as molecular sponges are negatively correlated with the corresponding miRNA in bone formation by upregulating downstream targets, but some experiments also suggest that circRNA can be positively correlated with the corresponding miRNA. [35] In terms of circRNA bone formation regulation, Zhang et al. found that miR-335-5p can promote bone formation differentiation by downgrading the Wnt antagonist Dickkopf-1 (DKK 1) [16] Many studies have shown that usually overexpression of circRNA can promote bone formation, because miR-335-5p can promote bone formation, according to the theory of microRNA (miRNA), we can promote the role of silent circRNA to explore the mechanism and target between mirRNA and circRNA. However, there are currently rare studies on miRNA associated with jaw defects at the circRNA level. Therefore, in the future, we can regulate miRNA through circRNA, a pathway of bone marrow mesenchymal stem cells, hoping to have a deeper understanding of the mechanism of bone differentiation, supplemented by cell membrane technology [36] Finally, applied to the treatment of clinically related bone defect disease [37].

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