

# Functions of microRNAs in osteoporosis

D.-W. GE<sup>1</sup>, W.-W. WANG<sup>2</sup>, H.-T. CHEN<sup>1</sup>, L. YANG<sup>3</sup>, X.-J. CAO<sup>1</sup>

<sup>1</sup>Department of Orthopedics, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

<sup>2</sup>Department of Gynecology and Obstetrics, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

<sup>3</sup>Department of Orthopedics, Nanjing First Hospital, Nanjing Medical University, Nanjing, China

*Dawei Ge and Weiwei Wang contributed equally*

**Abstract.** – Osteoporosis (OP) is a kind of disease with a 25% incidence, characterized by the bone mass loss, bone microstructure damage, increased bone fragility, and easy fracture. miRNA plays an important regulatory role in the process of bone remodeling, especially in the regulation of differentiation and function of osteoblasts and osteoclasts, and the development and progression of OP and other bone diseases. In the future, it is expected to delay the bone loss and promote the bone remodeling via the overexpression or inhibition of specific miRNAs in specific tissues, thereby treating OP.

Key Words:

microRNAs, Osteoporosis, Osteoblasts, Osteoclasts, Genes.

## Introduction

Osteoporosis (OP) is a kind of disease with a very high incidence, characterized by the bone mass loss, bone microstructure damage, increased bone fragility and easy fracture, etc. The mechanism of osteoporosis is mainly the excessive bone resorption caused by the increased osteoclast and excessive activation, and the decreased bone formation caused by the decreased osteoblast and functional defects.

Primary osteoporosis (POP) is divided into two types, postmenopausal osteoporosis (PMOP) and senile osteoporosis (SOP), known as type I and type II OP, respectively<sup>1</sup>. In PMOP, bone loss is mainly related to the increased osteoclast activity; bone resorption is greater than bone formation, and bone turnover is increased, leading to the bone mass loss and bone destruction. In SOP, bone loss is mainly related to the osteoblast function and activity defects; bone formation is decreased, and the osteoclast function is generally normal.

## microRNA

microRNA is a kind of short (about 18-25 nt) non-coding RNA, which performs the post-transcriptional control via inhibiting or degrading the target genes. Most miRNAs are highly conserved, and the regulation of miRNA in the development and progression of OP in different target cells has the time sequence and tissue specificity. Mature miRNA has the complementary sites to the 3'-end untranslated region (UTR) of the target mRNA, and they identify and combine with each other, so the translation cannot be performed, thus inhibiting the gene expression. miRNA plays an important regulatory role in the cell proliferation, differentiation and apoptosis, the development and progression of tumors and other physiological and pathological processes<sup>2</sup>.

## Mechanism of miRNA of Regulating Target Genes

miRNA negatively regulates the expression of target genes at the post-transcriptional level through two different mechanisms. When miRNA is almost completely paired with mRNA that encodes proteins, miRNA and mRNA transcripts form the miRNA-associated polypeptide RNA-induced silencing complex (miRISC), and mRNA in the target genes will be cut and further degraded by nuclease. This gene silencing mechanism mediated by miRNA is more common in plants. However, miRNA in most mammals does not lead to the degradation of target mRNA, but regulates the gene expression via another mechanism. These miRNAs form the complex similar to RISC through the incomplete base pairing and 3'-end UTRs, which inhibit the gene translation at the post-transcriptional level and reduce the protein expression level of its target genes, but its mRNA level is almost not affected.

### ***Mechanism and Regulation of Bone Remodeling***

Bones are continually remodeled during the whole life of human body to maintain a dynamic balance. This process mainly includes the bone resorption of osteoclasts for damaged bone tissues and new bone formation of osteoblasts. The process of bone remodeling is shown in Figure 1. Hematopoietic stem cells form the osteoclasts eventually via the osteoclast precursors to absorb the bone matrix and minerals; monocytes prepare the surface of bone tissue adsorption for the osteoblasts, and then osteoblasts secrete the bone matrix, followed by matrix mineralization and differentiation of some osteoblasts into bone cells, thus the process of bone remodeling is completed.

### ***Regulatory role of miRNA in the Development and Progression of OP in Different Target Cells***

Studies have shown that miRNA plays an important regulatory role in the process of bone remodeling, especially in the regulation of differentiation and function of osteoblasts and osteoclasts, and the development and progression of OP and other bone diseases<sup>3-5</sup>. In recent years, more and more researchers have focused on the mechanism of miRNA in the development and progression of OP, so as to find the effective gene therapy to treat or delay OP. The regulation of miRNA in the development and progression of OP in different target cells is reviewed now.

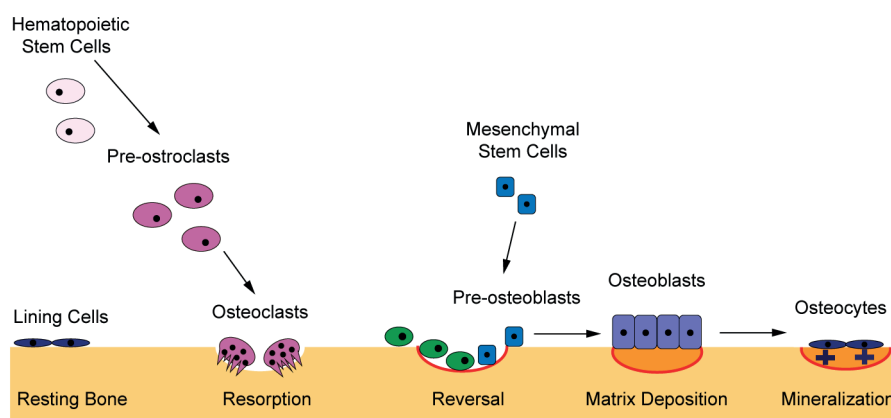
### ***Role of miRNA in Osteoblasts***

Osteoblasts are the main functional cells of bone formation, responsible for the synthesis, secretion and mineralization of bone matrix. Bones

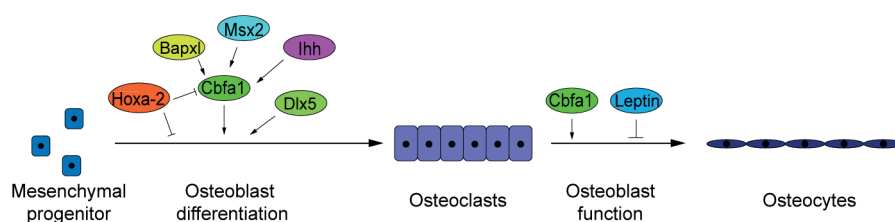
are continually remodeled, and the bone remodeling process includes the attachment of osteoclasts to the old bone region, secretion of acidoid to dissolve minerals, secretion of proteases to digest the bone matrix, and formation of the bone resorption lacunae. After that, the osteoblasts migrate to the absorption site and secrete bone matrix, and then the bone matrix mineralizes and forms new bones. The balance between osteolysis and osteogenesis is the key to maintaining normal bone mass. Osteoblasts undergo four stages during the osteogenesis: osteoblast proliferation, extracellular matrix maturation, extracellular matrix mineralization and osteoblast apoptosis. Many factors can adjust these stages, ultimately regulating the bone formation<sup>6</sup>. The precursor differentiation of osteoblasts, the mechanism of bone formation and the regulation process are shown in Figure 2.

With the aging of body, the balance between bone formation and bone resorption is broken in both male and female, due to the decline in the number of osteoblasts and decreased activity, leading to OP and other bone diseases with decreased bone mass. The external mechanism of this process may be the age-related changes in bone tissue microenvironment, such as hormone levels and growth factors; the internal mechanism is mainly the osteoblast senescence<sup>7</sup>. Recently, more and more studies have shown that the regulatory role of miRNA cannot be neglected in the process of osteoblast proliferation, differentiation and apoptosis.

Inose et al<sup>8</sup> used the micro-needle chip to screen a kind of miRNA (miR-206) highly expressed in the osteoblast of OP patients, and used dual-luciferase reporter gene system to confirm the target gene of miR-206, mRNA-Cx43. Cx43 is a kind



**Figure 1.** Mechanism and regulation of bone remodeling.



**Figure 2.** The precursor differentiation of osteoblasts, the mechanism of bone formation and the regulation process.

of gap junction protein between osteoblasts. The up-regulation of miR-206 in osteoblasts of OP patients inhibits the expression of Cx43 protein, thus affecting the function of osteoblasts.

In the development and progression of OP, oxidative stress is generally recognized as a very important factor, and uncontrolled oxidative stress damages the bone formation and induces bone mass loss. FoxO family plays a very important role in the protection of cells from oxidative stress, and FoxO1 is particularly critical in the bone tissue. FoxO1 activation can promote the osteoblast proliferation and differentiation, and can inhibit the osteoblast apoptosis. Kim et al<sup>9</sup> found that miR-182 in OP patients is significantly up-regulated compared with that in normal population. The up-regulated miR-182 inhibits the expression of FoxO1, thus destroying the protective system of oxidative stress, which promotes the osteoblast apoptosis and inhibits the proliferation and differentiation of osteoblasts.

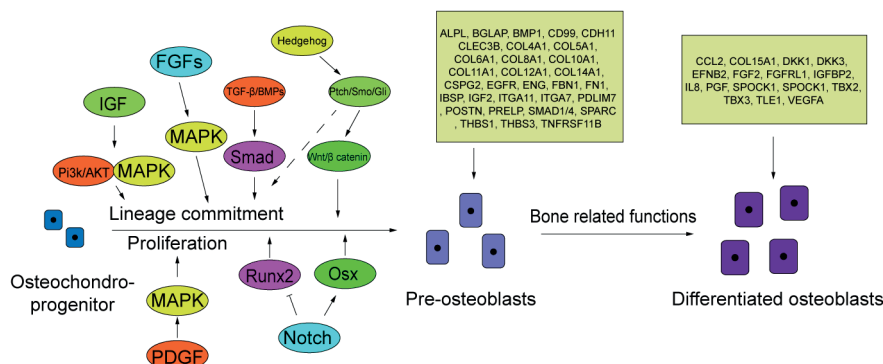
Wang et al<sup>10</sup> detected the miRNAs in femoral tissues of adults in different age groups and found that the expression of miR-214 is significantly correlated with the age. The expression of miR-214 increases with age, and the expression of miR-214 was found to be negatively correlated

with BGLAP and ALP that are closely related to the bone formation. The animal experiment was also performed by researchers and the experimental results showed that miR-214 was highly expressed in osteoblasts of mice receiving bilateral ovariectomy (OVX), suggesting that miR-214 can inhibit the osteoblast activity and matrix mineralization, etc. It was found in miRBase that miR-214 has the binding fragment to the 3'-end UTR of ATF4, and the target gene of miR-214 was confirmed combined with the dual-luciferase assay: ATF4. According to the study, it is expected to ease and improve the development and progression of OP via the *in vivo* inhibition of miR-214 expression in the future.

### Role of miRNA in Osteoclasts

Osteoclasts consist of multinucleated giant cells (MNGC), mainly distributed on the surface of bone and around the bone vessel channel. Osteoclast is a component of bone tissues, acting as the bone resorption. Osteoclasts correspond to osteoblasts in the function. They cooperate each other and play important roles in the formation and development of bones<sup>11</sup>.

Before performing the bone resorption, the osteoclasts undergo a process from precursor cells



**Figure 3.** The specific osteogenic differentiation process of MSCs and the regulation of this process.

to mature osteoclasts. Then the mature osteoclasts are attached to the bone surface to produce an isolated microenvironment between the bone tissue and the cell, thus degrading the organic matter and inorganic matter of bone tissue<sup>12</sup>.

The regulatory role of miRNA in the process of bone resorption of osteoclasts has been confirmed by more and more data; some miRNAs play roles in promoting the osteoclast differentiation, proliferation and activity, thus promoting the development and progression of OP; some miRNAs can promote the osteoclast apoptosis, thus delaying the bone loss in OP patients. These provide a theoretical basis for the gene target therapy of OP.

Recently, Wang et al<sup>13</sup> found that the survival rate of osteoclasts in miR-9 and miR-181a-knockout mice was higher than that in wild-type mice. The subsequent dual-luciferase reporter gene system experiment and Western Blot experiment confirmed the target gene of miR-9 and miR-181a: Cbl. Cbl protein is an important E3 ubiquitin ligase involved in the bone resorption, which has been widely recognized as the target gene of miR-9 and miR-181a. Cbl can regulate the osteoclast apoptosis through promoting the ubiquitin-dependent degradation of pre-apoptotic gene Bim. Bim is a member of BCL-2 protein family, and Bim gene has been recognized as an important pre-apoptotic gene of osteoclasts.

Monocytes in the blood, as precursors of osteoclasts, can secrete a number of cytokines associated with osteoclastogenesis: IL-1, IL-6 and TNF- $\alpha$ . Therefore, monocytes have often been used as the target cell in the study on OP in recent years. Cao et al<sup>14</sup> performed the miRNA array and qRT-PCR for monocytes in blood of two groups of postmenopausal women with high bone mineral density and low bone mineral density, and found that miR-422a was significantly up-regulated in postmenopausal OP patients and five target genes negatively correlated with it were found: CBL, CD226, IGF1, PAG1 and TOB2.

miRNAs undergo a process from precursor miRNAs to mature miRNAs before performing the post-transcriptional control of target genes, and some enzymes play important roles during the miRNA maturation process. Sugatani et al<sup>15</sup> studied and found that miRNA-223 can promote the osteoclast differentiation and function through down-regulating the NFI-A level. Researchers silenced the key factors during the miRNA-223 maturation process via the small-interfering RNA technique *in vitro*: DGCR8, Dicer and Ago2, which reduced the expression of miRNA-223,

thereby inhibiting the osteoclastic differentiation and bone resorption. Researchers also found that Dicer-knockout mice showed a certain degree of osteosclerosis, and the *in vitro* and *in vivo* experiments have shown that Dicer and other factors play important roles in miRNA maturation and function, thus regulating the osteoclast resorption.

### **Role of miRNA in Mesenchymal Stem Cells**

Mesenchymal stem cells (MSCs) are a group of adult stem cells with self-renewal and multi-differentiation capacity. In different induction environments, it can differentiate into osteoblasts, adipocytes, chondroblasts and neuroblasts. Studies have shown that MSCs are precursor cells of osteoblasts and bone cells. Induction of directional differentiation of MSCs into bone tissues is of great significance in the treatment of bone mass loss and repair of bone defects<sup>16</sup>.

Some key molecules or pathways that play definite roles in promoting osteogenic differentiation of MSCs have been found at present, such as bone morphogenetic protein (BMP) family, Runx2, ALP, Cadherin family and Wnt signaling pathway. These molecules or pathways interact and contact one another, constituting a huge regulatory network<sup>17</sup>.

MSCs can not only differentiate into osteoblasts, but also regulate the osteoclastogenesis through the expression of RANKL and OPG, etc.<sup>18</sup>. Experimental data show that MSCs can express RANKL to promote osteoclastogenesis, and also express OPG receptors and secrete OPG protein to inhibit osteoclastogenesis<sup>19,20</sup>. The specific osteogenic differentiation process of MSCs and the regulation of this process are shown in Figure 3.

Eskildsen et al<sup>21</sup> discovered a kind of miRNA significantly down-regulated during the osteogenic differentiation of MSCs, miR-138, through miRNA chip and qRT-PCR. Target gene prediction test confirmed the target gene of miR-138: FAK (a kind of kinase that promotes osteogenic differentiation). Scholars used anti miR-138 to inhibit the expression of miR-138, so as to promote bone formation, which may become an effective treatment strategy for OP in the future.

Many inflammatory factors, such as tumor necrosis factor (TNF)- $\alpha$ , have been proved to inhibit the osteogenic differentiation of MSCs in OP caused by estrogen deficiency. Yang et al<sup>22</sup> applied chip and qRT-PCR and found that the expression of miR-21 is significantly lower in MSCs in OVX mice, and the subsequent experiments showed

that the down-regulated expression of miR-21 is due to the TNF- $\alpha$  inhibition. miR-21 can promote the osteogenic differentiation of MSCs through inhibiting the expression of target gene *Spry1*. *Spry1* is the conserved inhibitor of receptor tyrosine kinase (RTK) pathway, which negatively regulates multiple RTK signaling pathways, such as FGF and MAPK signaling pathways. *Spry1* also plays a negative regulatory role in the osteogenic differentiation of MSCs.

*Runx2* is a kind of osteoblast-specific gene, which has been widely studied as a star molecule in OP. Li et al<sup>23</sup> discovered a new miRNA (miR-2861) that can reduce the degradation of *Runx2* and promote osteogenic differentiation of MSCs in mice through inhibiting the expression of HDAC5, and they also found that the expression of miR-2861 is reduced in POP patients.

The different species, different target cells and different regulation of target genes are likely to cause a certain differences of the same miRNA in functions. For example, the high-expression miR-210 can promote the osteogenic differentiation of BMMSC line, ST2, in mice. In contrast, miR-210 is down-regulated in the osteogenic differentiation of human MSCs, and the inhibition of miR-210 can promote the osteogenic differentiation of human MSCs.

## Conclusions

In normal cells, the expressions of various miRNAs are in a state of maintaining the balance of cell proliferation, differentiation, apoptosis and other physiological processes. The same miRNA may have a variety of different target gene mRNAs, so the disorder of miRNA expression can cause a series of serious intracellular dysfunction<sup>24</sup>. The research on miRNA has focused on the development and progression of tumors, but more and more research data have shown that miRNA also plays an important role in the regulation of pathogenesis of various bone diseases; in particular, the correlation between miRNA and development and progression of OP has become a research hotspot. It is worth noting, however, that the important role of miRNA in regulating the differentiation and function of osteoblasts and osteoclasts is only the first step in clinical practice, and more studies are needed to put miRNA-based therapies into clinical practice successfully.

Current studies have shown that the overexpression or inhibition of specific miRNAs in a tissue-specific way has a certain therapeutic ef-

fect in the tumor animal model, and some miRNA-based treatment methods have been in the clinical trial stage<sup>25, 26</sup>. The miRNA-based treatment method of OP has not been in the clinical stage yet, so more studies are needed. In the future, it is expected to delay the bone loss and promote the bone remodeling via the overexpression or inhibition of specific miRNAs in specific tissues, thereby treating OP.

## References

- 1) KENDLER D. Osteoporosis: therapies now and in the future. *Climacteric* 2011; 14: 604-605.
- 2) YATES LA, NORBURY CJ, GILBERT RJ. The long and short of microRNA. *Cell* 2013; 153: 516-519.
- 3) LIU J, LI Y, LUO M, YUAN Z, LIU J. MicroRNA-214 inhibits the osteogenic differentiation of human osteoblasts through the direct regulation of baculoviral IAP repeat-containing 7. *Exp Cell Res* 2017; 351: 157-162.
- 4) BAE Y, YANG T, ZENG HC, CAMPEAU PM, CHEN Y, BERTIN T, DAWSON BC, MUNIVEZ E, TAO J, LEE BH. MiRNA-34c regulates Notch signaling during bone development. *Hum Mol Genet* 2012; 21: 2991-3000.
- 5) FU HL, PAN HX, ZHAO B, DONG BC, SHAO L, FU GS, WANG Q, LI M. MicroRNA-100 inhibits bone morphogenetic protein-induced osteoblast differentiation by targeting Smad1. *Eur Rev Med Pharmacol Sci* 2016; 20: 3911-3919.
- 6) DUCY P, SCHINKE T, KARSenty G. The osteoblast: a sophisticated fibroblast under central surveillance. *Science* 2000; 289: 1501-1504.
- 7) MARIE PJ, KASSEM M. Osteoblasts in osteoporosis: past, emerging, and future anabolic targets. *Eur J Endocrinol* 2011; 165: 1-10.
- 8) INOSE H, OCHI H, KIMURA A, FUJITA K, XU R, SATO S, IWASAKI M, SUNAMURA S, TAKEUCHI Y, FUKUMOTO S, SAITO K, NAKAMURA T, SIOMI H, ITO H, ARAI Y, SHINOMIYA K, TAKEDA S. A microRNA regulatory mechanism of osteoblast differentiation. *Proc Natl Acad Sci U S A* 2009; 106: 20794-20799.
- 9) KIM KM, PARK SJ, JUNG SH, KIM EJ, JOGESWAR G, AJITA J, RHEE Y, KIM CH, LIM SK. MiR-182 is a negative regulator of osteoblast proliferation, differentiation, and skeletogenesis through targeting FoxO1. *J Bone Miner Res* 2012; 27: 1669-1679.
- 10) WANG X, GUO B, LI Q, PENG J, YANG Z, WANG A, LI D, HOU Z, LV K, KAN G, CAO H, WU H, SONG J, PAN X, SUN Q, LING S, LI Y, ZHU M, ZHANG P, PENG S, XIE X, TANG T, HONG A, BIAN Z, BAI Y, LU A, LI Y, HE F, ZHANG G, LI Y. MiR-214 targets ATF4 to inhibit bone formation. *Nat Med* 2013; 19: 93-100.
- 11) BOYLE WJ, SIMONET WS, LACEY DL. Osteoclast differentiation and activation. *Nature* 2003; 423: 337-342.
- 12) TEITELBAUM SL. Bone resorption by osteoclasts. *Science* 2000; 289: 1504-1508.

- 13) WANG S, TANG C, ZHANG Q, CHEN W. Reduced miR-9 and miR-181a expression down-regulates Bim concentration and promote osteoclasts survival. *Int J Clin Exp Pathol* 2014; 7: 2209-2218.
- 14) CAO Z, MOORE BT, WANG Y, PENG XH, LAPPE JM, RECKER RR, XIAO P. MiR-422a as a potential cellular microRNA biomarker for postmenopausal osteoporosis. *PLoS One* 2014; 9: e97098.
- 15) SUGATANI T, HRUSKA KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. *J Biol Chem* 2009; 284: 4667-4678.
- 16) BIELBY R, JONES E, MCGONAGLE D. The role of mesenchymal stem cells in maintenance and repair of bone. *Injury* 2007; 38 Suppl 1: S26-S32.
- 17) ACIL Y, GHONIEM AA, WILTFANG J, GIERLOFF M. Optimizing the osteogenic differentiation of human mesenchymal stromal cells by the synergistic action of growth factors. *J Craniomaxillofac Surg* 2014; 42: 2002-2009.
- 18) WILTON JM. Denosumab: new horizons in the treatment of osteoporosis. *Nurs Womens Health* 2011; 15: 249-252.
- 19) GORI F, HOFBAUER LC, DUNSTAN CR, SPELSBERG TC, KHOSLA S, RIGGS BL. The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. *Endocrinology* 2000; 141: 4768-4776.
- 20) GIULIANI N, BATAILLE R, MANCINI C, LAZZARETTI M, BARILLE S. Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. *Blood* 2001; 98: 3527-3533.
- 21) ESKILDSEN T, TAIPALEENMAKI H, STENVANG J, ABDALLAH BM, DITZEL N, Nossent AY, Bak M, Kauppinen S, Kassem M. MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. *Proc Natl Acad Sci U S A* 2011; 108: 6139-6144.
- 22) YANG N, WANG G, HU C, SHI Y, LIAO L, SHI S, CAI Y, CHENG S, WANG X, LIU Y, TANG L, DING Y, JIN Y. Tumor necrosis factor alpha suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. *J Bone Miner Res* 2013; 28: 559-573.
- 23) LI H, XIE H, LIU W, HU R, HUANG B, TAN YF, XU K, SHENG ZF, ZHOU HD, WU XP, LUO XH. A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest* 2009; 119: 3666-3677.
- 24) KASINSKI AL, SLACK FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. *Nat Rev Cancer* 2011; 11: 849-864.
- 25) TIEMANN K, ROSSI JJ. RNAi-based therapeutics-current status, challenges and prospects. *Embo Mol Med* 2009; 1: 142-151.
- 26) KAPINAS K, DELANY AM. MicroRNA biogenesis and regulation of bone remodeling. *Arthritis Res Ther* 2011; 13: 220.