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Osteogenesis Through miRNA: The Unique Role of Signaling Proteins.

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ABSTRACT

This review addresses some of the main aspect of different stem cell line commitment to osteogenic lineage by signaling proteins and transcription factors at the micro RNA (miRNA), level. Bone remodeling which occurs in basic multi cellular unit of bone which is characterized by bone formation and bone resorption. The present evidence signifies that miRNAs, which is widely recognized as a family of short non-coding RNAs, are the key post-transcriptional repressors of gene expression and many novel miRNAs have been verified to play vital roles in the regulation of osteogenesis, disclosing how they interact with signaling molecules to control these processes. This review summarizes the current knowledge of the roles of miRNAs in regulating osteogenesis regulated by signaling molecules and transcription factors.

Keywords: miRNA, Osteogenesis, Signaling Proteins

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INTRODUCTION

The human genome comprises less than 2% of protein coding genes, however, the rest is made up of non-protein coding DNA [1]. The majority of transcription output in human genome is contributed by non-coding ribonucleic acid (RNA) [2]. Non coding RNAs, involves transfer RNA and ribosomal RNA as well as long non-coding RNAs, small interfering RNAs (si RNAs) and miRNAs [3] as depicted in figure 1.

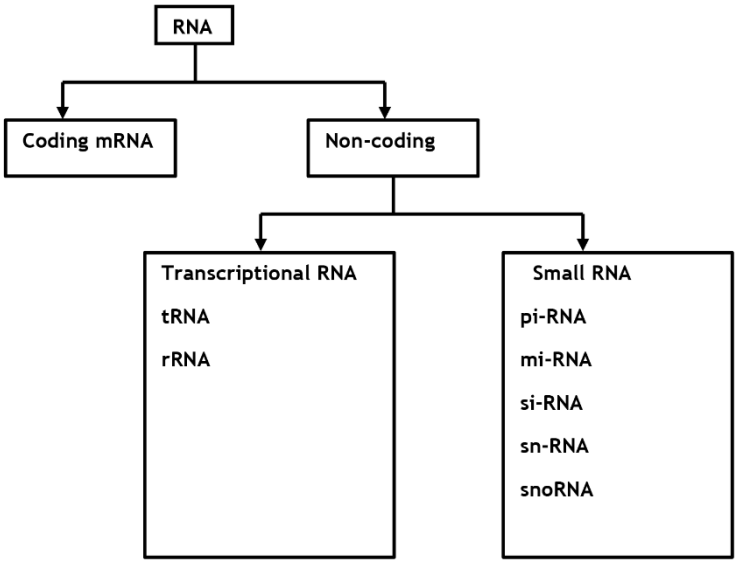

Figure 1: Classification of RNA

In 1993, the first miRNA, lin-4 was discovered in the Ambros and Ruvkun labs. Lin-4 is crucial for the development of nematode caernohabditiselegans[4,5].This was followed by the discovery of second miRNA let-7, which was also identified in the nematode C.E. in 2000 [6]. Soon after the discovery of let-7 in animals, it was identified in human beings and in animals [7]. The various biological functions of miRNAs such as growth, angiogenesis, proliferation and differentiation via down regulating one-third of all human genes at the stage of translation. In human genetic system, miRNA comprises 1 to 3% of total genes in the human body [8].

miRNA biology

In the nucleus, miRNA are translated into primary miRNAs (pri-miRNAs). It is made up of more than 1000 nucleotides. Then, pri-miRNAs undergoes cleavage with the help of enzyme DroshaInase III [9] and converted into precursors (pre -miRNAs). This precursor miRNAs comprises of 60-100 nucleotides [10]. This in turn follows, the transportation of pre-miRNAs from nucleus to cytoplasm with the assistance of Exportin S. In cytoplasm, it undergoes again cleavage to form amiRNA duplex in the presence of a second RNase III enzyme Dicer [11]. Basically, miRNA duplex constitutes about 15 to 22 nucleotides in length. Subsequently, duplex unwinds into mature miRNA and passenger miRNA. The final effect of mRNA translation and degradation results, after incorporation of mature miRNA into RNA-induced silencing complex (RISC) [12]. On the other hand, the unique miRNA subgroup, mirtrons, depend directly on splicing activity, and bypassing the DROSHA cleavage step [13, 14]. miRNA can perform various rolesin the formation of the human musculoskeletal system.The biogenesis is given in figure 2.

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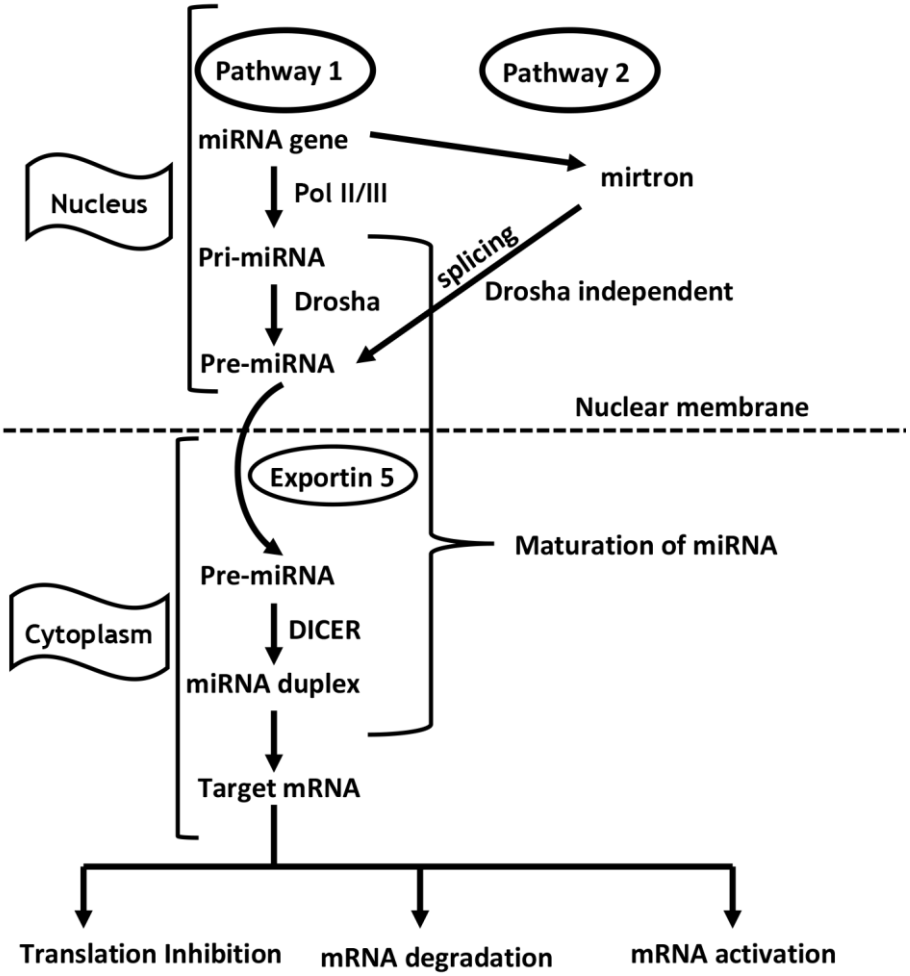

Figure 2: Micro RNA Biogenesis

These miRNAs are the next plausible frontier of the research focusing on prevention and treatment of osteoporosis and bone injuries.

Bone

Bones are the building blocks of the skeletal framework, which further acts as the basic structure on which the human body is constructed. Apart from providing structural integrity and thus regulating bodily movement, bone acts as a storage house for minerals and fats like triglycerides. It plays a key role in mineral hemostasis (calcium and phosphorus). The internal organs find a protective barrier in the skeletal framework. Bone also participates in the endocrine regulation of energy metabolism. Production of blood cells (haematopoiesis) are done by bone marrow, which is an integral part of the bones[15] Thus bones are a boon to humans in multiple ways. Bone is constructed in such a manner that it is constantly subjected to "remodeling" mechanism. This is definitely to preserve its structural integrity and strength. Osteoblasts and osteoclasts, the two contrasting forces, regulate remodeling in the bone microenvironment [16].Osteoblasts apart from being key regulators in osteoclast differentiation and resorption, also play a prominent role in the initiation, early stage bone mineralization and bone remodeling [17]. The bone remodeling process is checked and regulated by a number of ancillary factors such as hormones, cytokines and growth factors. Recent literature have highlighted the prominent role played by the close interactions between bone and immune cells such as cytokines in bone metabolism[18].

Bone remodeling

Bone remodeling which is a major regulatory mechanism, occurs mostly during adulthood. The bone homeostasis is maintained by two processes namely bone formation and resorption. In detail, bone

remodeling involves basically the breaking down of the old bones followed by deposition of the inorganic matrix and the further apposition of the proteinaceous matrix [15].This continuous mechanism, mainly focused on cortical and trabecular sites, controls cellular activity through the life-span of the bone [19-22]. Bone remodeling revolves around two fundamental cells, namely osteoblasts which are induced by mesenchymal cells and osteoclasts, induced by hemopoietic stem cells (HSCs) and are closely interconnected by regulatory proteins which helps in the formation of new and the resorption of old bone[23,24].

According to Bellido *et al.*, 2014 [25], bone remodeling is the balance of resorption and formation. In the remodeling mechanism, the coupling process of osteoblasts and osteoclasts occurs at definite sites, called basic multicellular units (BMU). The BMU in corticaland trabecular bone differs morphologically, although the mechanism is the same. The larger surface area of the trabecular bone helps in prompt remodeling than the cortical bone [26].The precursors of remodeling are supplied and distributed by bone marrow and blood stream [22]. Osteoprogenitor synthesizes osteoblasts which further makes up the lining cells, of which some are stored as osteocytes [27]. The active osteoclast is characterized by its ruffled border with deep plasma membrane folds where the active bone resorption takes place [28].

Osteoblasts and osteoclasts regulate the development, maintenance and repair of bone thereby regulating the bone remodeling process [29]. The cuboidal osteoblasts, originating from osteoprogenit or mesenchymal stem cells (MSCs), are the key players in bone formation [30].The major functions performed by osteoblasts are bone formation, osteoclast differentiation and expansion of HSCs. The bone formation is characterized by the synthesis of Extracellular matrix (ECM) proteins such as Type I collagen (COL1), osteocalcin (OCN), osteopontin (OPN) and bone sialo protein (BSP) on which osteoid is deposited [31,32].Osteoblasts generate increasing levels of bone specific alkaline phosphatase (ALP) mainly on the outer membrane, which catalyzes mineralization. Osteoblasts secrete proteins into the blood, the proteins signaling bone formation [33].

Mechanosensory cells belonging to the osteoblastic family such as osteocytes, osteoblastic line age and osteoblasts play a pivotal role in the regulation and adaptation of bone [34, 35].A few osteoblasts which are converted to osteocytes, become engulfed in the bone ECM and in turn branches with the nearby osteocytes [36]. Osteocytes surveil mechanical stress (i.e.pressuresorcracks) in the bone and further generate prostaglandins. They initiate osteoclasts into dissolving the bone[37].Remodeling process actually starts with the formation of lining cells from osteoblasts [38].

Apart from regulating the flow of calcium to and from the bone, the osteoblast lineage cells which form the ECM of bone, acts as a warehouse for a variety of growth factors. Also to be noted is the structural integrity and strength provided by these cells to the bone [39]. They also signal peculiar proteins which induces the osteoclasts [34]. The non-collagenous proteins and type I collagen secreted by osteoblasts controls bone mineralization [28].

Mechanism of Osteoblastogenesis

The process of osteoblastogenesis from mesenchymal progenitors to a mature bone forming cell involves 3 stages. First stage is commenced by the upregulation of chondrocyte master transcription factor sex-determining region Y (SRY)-box 9 (SOX9) in the mesenchymal progenitors[40].Thereafter, the osteoblast master transcription factor runt-related transcription factor 2 (RUNX2) transform the proliferating precursor cells to the osteoblast lineage resulting in the formation of osteochondroprogenitors. Subsequently SOX9 is downregulated and thereby leads to the formation of RUNX2-positive osteoprogenitors [41]. At this stage, Osterix (OSX), a second osteoblast master transcription factor along with mediates the expression of COL1 and ALP [42]. This leads to the formation of preosteoblasts also known as immature osteoblasts. Additionally, the transcription factor ATF-4 also plays a key role in osteoblast differentiation. So besides the transcription factors, certain genes are essential for the activation of pre-osteoblast proliferation (eg: c-Fos, c-Jun and c-myc) and cell cycle progression (eg: histones and cyclines) are expressed along with the genes of the growth factors such as fibroblast growth factor, insulin-like growth factor-1, transforming growth factor, BMPs, cell adhesion proteins and COL1 [43]. The second stage starts with the maturation and organization of the bone ECM following the proliferatory phase. This stage is characterized by the continuation of collagen synthesis and cross-link maturation. Gene responsible for this phase is ALP [43]. Under alkaline conditions, the crucial role of ALP in bone calcification is characterized by hydrolysis of ester bonds in organic phosphate

compounds. This infers that high ALP activity corresponds to ECM formation before moving to the mineralization phase [43].

The third stage is characterized by the downregulation of the genes that aid in proliferation as well as upregulation of genes involved in mineralization phase. During mineralization phase, maximal expression of genes encoding the noncollagenous proteins such as OCN, OPN and BSP occurs. In the final phase, collagenases are elevated and 50-70% of osteoblasts undergo apoptotic activity [44].Different osteogenic markers in various phases of bone formation as pictorically shown below in figure 3:

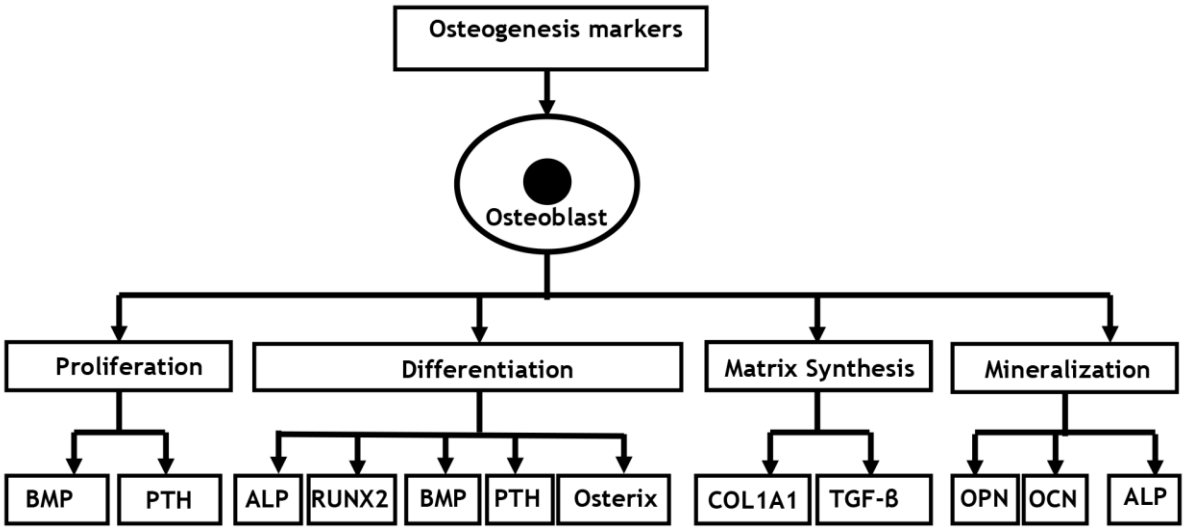

Figure 3: Osteogenic markers during proliferation, differentiation, matrix synthesis and mineralization

SIGNALING PROTEINS AND ITS miRNA REGULATION IN OSTEOGENESIS

RANK/RANKL/OPG signaling

RANKL, a type 2 ligand protein of TNF ligand family is an essential instigator for osteoclast differentiation. This factor exists as a homotrimeric protein and is expressed as a membrane - bound protein on the surface of osteoblasts, osteocytes, marrow stromal cells, activated T cells, and B-cells. M-CSF, along with most osteotropic factors such as IL-1, IL-11, prostaglandin E2 and 1,25-(OH)2D3, induces osteoclast formation by binding to marrow stromal cells, which in turn express increased levels of soluble or membrane forms of RANKL [45]. OPG, which is a secreted member of TNF receptor family, is produced by osteoblasts, marrow stromal cells similar to RANKL molecule. Its main action is to inhibit osteoclast formation and activity. OPG, the name itself signifies protector of bone.

In small cell lung cancer cell line, miR-335 inhibits bone metastases by the reduction of IGFIR and RANKL expression [46]. In MC3T3-E1, miR-17 and miR-20a inhibits glucocorticoid induced osteoclastogenesis by suppressing RANKL expression [47]. An *invitro* study in giant cell tumor showed that miR-106b inhibits osteoclastogenesis by RANKL expression [48].The over expressed miR-20a in dexamethasone induced osteoblasts leads to down regulation of RANKL expression, consequently suppressing bone resorption [47]. In Dicer-deficient cells, on treating with RANKL, the expression of miR-155 was downregulated [49]. It was noted that decreased release of osteoclasts resulted in miR-155 knockout mice. Thus miR-155 could inhibit bone destruction [50]. In THP-1 cells, the overexpression of miR-145 results in downregulation of OPG mRNA expression and upregulation of RANK and RANKL [51].In patients suffering from postmenopausal osteoporosis, the miR-503 inhibited bone resorption by directly RANK [52].

BMP signalling

Bone morphogenetic proteins (BMPs) are the members of transforming growth factor-β (TGF-β) superfamily. Signaling is mainly regulated by R – SMADs (SMAD1, SMAD 5, and SMAD 8) and Co-SMAD

(SMAD4).The synergic action between BMPs and their receptors results in the phosphorylation of SMADs. The activated R-SMADs combine with SMAD4 and thereby regulate osteogenesis.RUNX2 is crucial in the activation of SMADs by BMPs as well. RUNX2, belonging to the RUNX transcriptional factors family, which modulates osteoblast differentiation by regulating theosteogenic gene such as OPN, BSP, and OCN [53]. As shown in vitro study of mouse calvarial osteoblasts, miR-542-3p inhibits osteogenesis through the suppression of BMP-7 [54]. In ST2 cells, under activity of miR-125b enhanced BMP induced osteogenesis [55]. In C2C12 cells, miR-133 and miR-135 synergistically acted to induce osteogenic differentiation [56, 57]. In MC3T3-E1 cells, they observed that miR-141 and miR-200a are involved in BMP-2 induced pre-osteoblast differentiation by regulating the expression of Dlxs [58]. In MC3T3-E1 cells, over expression of miR-208 enhanced attenuated BMP-2 induced osteogenic differentiation [59]. MiR-20a directly bonds to 3 VTR of BMP-2 promotes osteogenic differentiation by the upregulation of BMP/RUNX2 signaling [60]. The over expression of miR-210 activated BMP4 induced preosteoblast differentiation [61]. MiR-26a inhibits osteoblast differentiation by targeting SMAD1 [62]. The finding by Li et al that 22 miRNAs down-regulated in the BMP-2-induced osteoblast differentiation points to the possibility of the inhibitory role played by miRNAs towards the function of osteoblast differentiation factors [63, 64]. Sun et al. observed that miR-106b expression was down-regulated slightly during BMP2-mediated C2C12 osteogenic differentiation [63, 65]. RUNX2 and SMADs1/5 are mandatory for osteogenic differentiation. Added to this, miR-133 downregulates RUNX2.Thus, miR-133 inhibits the osteogenic differentiation of C2C12 that had been induced by BMP-2 [63]. According to Mizuno et al, miR-125b has a modulatory effect on the osteogenic differentiation of ST2, a MSC line. miR-125b expression gradually downregulated during BMP4-induced ST2 proliferation [61]. miR-26a which acts as an inhibitor of *Smad1* [66], further proved in human ADSCs [62],results in the inhibition of late stage osteoblast differentiation. In mouse bone marrow stromal cells, a down-regulation in BMP2-induced osteoblast differentiation resulted, when the expression of miR-2861 was silenced [64]. In other words, miR-2861 upregulated during osteoblast formation on induction by BMP2 [67]. According to Li et al, miR-17-5p and miR-106a helps in maintaining the balance between osteogenic and adipogenic differentiation through the modulation of BMP2 [68]. MiR-106b-5p and miR-17- 5p hamper osteogenic differentiation through the regulation of SMAD5, which forms part of BMP2/SMAD pathway [69]. Modulation of miR-140-5p expression levels creates a controlling impact on BMP2. In hMSCs, the up-regulation of BMP2, bone morphogenic protein receptor type II (BMPR2), type 1B (BMPR1B) and SMAD5 resulted through the impeding of miR-140-5p. miR-140-5p inhibition further led to the upregulation of osteogenesis modulators like*RUNX2*, *ALP*,*OCN* and *OPN* in undifferentiated hMSCs. Further, miR-140-5p inhibition led to osteoblast generation in the ALP staining assay which was evident from the upregulation of ALP levels [53]. In ST2 stromal cells, increase in the miR-3960 expression levels led to the upregulation of homeobox protein A2 (HOXA2) which further promoted BMP2-induced osteoblastogenesis [67]. BMP2 induced osteogenesis in C2C12 cells, which was evident from the upswing in the expression levels of osteoblast markers such as ALP, COL1A1, and OCN. Further, miR-214 expression gets downregulated during osteogenesis. Furthermore, the negative impact created by miR-214 on osteogenesis is reiterated by the increase in the levels of osteoblast markers such as ALP, COL1A1, and OCN due to the transfection of miR-214 inhibitors [70]. Luzi et al. (2008) proved that miR-26a controls late osteoblastogenesis in subcutaneous human adipose tissue by impacting SMAD1 transcription factor [61]. According to Yang et al (2012), miR-93 modulates osteoblast mineralization through the miR-93/Sp7 regulatory feedback loop [71]. miR-30 family repress BMP-2-induced osteoblastogenesis through the regulation of SMAD1 and RUNX2 [72].

According to Zeng et al 2012, miR-100 suppresses osteogenic differentiation of human adipose derived mesenchymal stem cells (hAMSCs) through the inhibition of BMP signaling by targeting the BMP receptor 2 (BMPR2). Members of the miR-30 family and miR- 135a downregulate RUNX2 expression. Apart from this, they interfere with BMP signaling by targeting the signal transducers SMAD1 and SMAD5 [73]. MiR-2861 is a miRNA that is predominantly expressed in bone and becomes upregulated during BMP2-induced osteoblast formation. Besides its important function as a positive regulator of RUNX2 signaling, miR-2861 has been associated with OP in humans. Two related adolescents with juvenile OP have been described to carry a homozygous mutation in pre-miR- 2861, leading to loss of mature miR-2861 in their bones [64].

TGF-β signaling

Canonical SMAD-dependent TGF-β signaling first binds to receptor type II and receptor type I, and then signaling transduces to their SMADs 2/3. Activated SMADs form a complex with SMAD 4 and then

translocate into the nucleus where they interact with other transcription factors to trigger target gene expression RUNX2. SMAD7 disrupts the activated SMAD2/3 to form a complex with SMAD4 [74, 75]. TGF- β1 would increase miR-145 expression and the up-regulated miR-145 thereby induced Wnt/ b-catenin signaling activation, which have been demonstrated in MSC [76].An in-vitro study in CSTBL/6- derived bone marrow cells revealed that miR-349 inhibits osteoclastogenesis through the suppression of TGF- β induced factor II. The increased level of miR-29b cause an inhibitory effect of TGF-β on osteoblast regulatory mechanism in primary fetal rat calvarial osteoblasts [77]. Some miRNAs like miR-210 promote osteogenesis by the inhibition of the activity of the TGF-β type I receptor [61].In bone marrow-derived ST2 stromal cells, osteoblastogenesis induced by BMP-4 increased due to the transfection of sense miR-210. This occurs specifically due to miR-210 inhibiting the TGF-β/activin signaling pathway by hampering Activin receptor type-1b gene(AcvR1b) [61]. MiR-29b stimulates osteogenesis by suppressing inhibitors of osteoblast differentiation, namely HDAC4, TGF-β 3, ACVR2A, CTNNBIP1 (catenin, beta interacting protein 1), and DUSP2 proteins by binding to target 3'-UTR sequences in their mRNAs. Thus, miR-29b promotes development of the osteoblast phenotype by suppressing both anti-osteogenic factors and bone extracellular matrix proteins [64].

WNT signalling

The role of Wnt pathways in bone remodeling process is very pathognomonic [26]. Wnts are considered as anabolic signaling protein that ameliorates the proliferation and differentiation of osteoblast progenitors [78-80]. Specific Frizzled (FZD) proteins and the low-density lipoprotein receptor related protein 5/6 (LRP-5/6) formed a receptor complex for Wnt ligand. The interaction results in release of β-catenin, which in turn regulates the transcription factor, Runx2 [81].In summary, we demonstrated that ossotide induced miR-145 up-regulation in hOBs cells. The up-regulated miR-145 promoted RUNX2 and OSX expression, which related to osteoblasts differentiation. Wntsignaling was also activated by ossotide via miR-145. All these results confirmed that miR-145 is a key target of ossotide in regulating osteoblasts differentiation [82]. Wang and Xu stated that miR-27 promotes osteoblast differentiation by modulating Wnt signaling in hFOB cell line [83]. MiR-29c promotes Wnt signaling in osteoblasts by targeting inhibitors which regulates matrix proteins [84, 85]. Zhang et al stated that miR-335-sp modulated osteogenic differentiation by specific down-regulation of Wntantagonist Dkk1 in MC3T3-E1 cell line [86]. An *invitro* study showed that miR-29a lessen bone loss by suppressing histone deacetylase 4 (HDAC4), which in turn activates the acetylation of β-catenin and thus enhances osteogenesis.miR-29 plays a modulatory role in osteoblast differentiation through the down-regulation of osteonectin expression via the canonical Wntpathway [84].The activation of miR-29a leads to down-regulation of key Wnt signaling antagonists, Dkk1, Kremen2, and sFRP2, thus activating osteoblast differentiation [87].miR-29 and miR-218 enhance osteoblast differentiation by regulating Wnt signaling [87].In MSCs, the miR-145 expression would enhance by TGF-β 1, thereby increase the activation of Wnt/β-catenin signaling [87].

TNF signalling

TNF-α activates the NF-κb pathway which inturn stimulated BMP-2 up-regulation results in the augmentation of RUNX2 and OSX expression, two pivotal regulators of the osteogenic differentiation [88]. Yang et al [89] specified that the downregulation of miR-21 may bestow the tumor necrosis factor-α induced suppression of osteogenesis in estrogen deficiency – induced osteoporosis

Notch signalling

In mammals, the canonical Notch signaling is comprised of five Notch ligands [Jagged1 and 2, and Delta-like (DLL) 1, 3 and 4] and four Notch receptors (Notch1-4) (3, 4). After binding, the Notch intracellular domain (NICD) is cleaved and released. This in turn relocates to the nucleus [90]. Later, the NICD binds with the transcriptional regulator of the CSL family to regulate downstream targets [91]. This is crucial in maintaining the undifferentiated state of MSCs [92-97]. The activation of Notch signaling in apical papilla stem cells. The osteogenesis mechanism was enhanced in SCAPs by miR-34a through Notch signaling by directly acting on Notch 2 and HES1 [98].

miRNA in RUNX2 regulation

MiR-23a-27a-24-2 cluster is downregulated by RUNX2 to promote osteogenesis [99]. MiR-204 and miR-211 has an inhibitory effect on RUNX2 expression in C3H10T12 and ST2 cells and in hMSCs [100]. MiR-335 over expression can inhibit osteogenesis in hMSCs [101]. MiR-29a suppresses the expression of HDAC4 could increase acetylation of RUNX2 and increase osteogenesis [102]. In human BMSCs, miR-15b enhances the osteoblast differentiation due to the upregulation of ALP and COL1 by targeting Smurf1, which has a great role to prevent RUNX2 from degradation [103]. MiR-2861, in mice elevated osteogenesis by suppressing HDAC5 which usually deacetylates RUNX2 [98].The regulation loop compressed of Runx2/miR-3960/miR-2861 enhances osteogenic differentiation by increasing the activity of ALP and OCN [104]. MiR-204 suppresses the expression of osteogenic genes such as RUNX2, OPN and OCN and finally decrease ALP activity and bone matrix formation [105,106]. Liao et al stated that miR-705 and miR-3077-5p suppressed differentiation by inhibiting HomeoboxA 10 and RUNX2 mRNA [107]. Liu et al stated that miR-338-3p inhibited the expression of osterix, which is the downstream target of RUNX2 in BMSCs [108].

miRNA in OSX regulation

MiR-125b acts as a negative regulator of osteogenesis by targeting OSX due to the reduction of ALP, COL1 and OCN[109]. Interestingly, miR-637 showed an inhibitory effect on osteoblasts. miR-637 inhibits OB differentiation via OSX [110]. Interestingly, studies in the past have shown that miR-145 modulates OSX protein expression. This was affirmed that OSX is a target of miR-145 [111]. OSX is crucial or skeletal morphogenesis, which is chemically a zinc finger transcription factor. miR-145 modulates osteoblast differentiation by targeting cbfb and OSX. They observed that the expression of RUNX2 and OSX were enhanced with the high level of miR-145,specifying that this miRNA is crucial in osteoblast [112].

The osteoblast differentiation can be regulated by several miRNAs which can act upon certain transcriptional factors and causes their upregulation and down regulation as mentioned in Tables 1 and 2.

Table 1: miRNAs causing upregulation during osteoblast differentiation

MicroRNA	Target	Reference
miR-210	VEGF	(Liu *et al.*, 2015)[113]
miR-216a	Cb1-mediated PI3K/Akt	(Li *et al.*, 2015b)[114]
miR-29a	Runx2	(Ko*et al.*, 2015)[115]
miR-20a	BMP/Runx2	(Zhang *et al.*, 2011)[116]
miR-2861	HDAC5	(Li *et al.*, 2009)[102]
miR-378	Caspase-3	(You *et al.*, 2014)[117]
miR-26a	SMAD1	(Luzi*et al.*, 2008)[62]

Table 2: miRNAs causing downregulation during osteoblast differentiation

MicroRNA	Target	Reference
miR-204	RUNX2	(Huang *et al.*, 2010)[98]
miR-3077-5p	RUNX2	(Liao *et al.*, 2013)[105]
miR-103a	RUNX2	(Zuo*et al.*, 2015)[118]
miR-34c	BMP-2	(Bae*et al.*, 2012)[119]
miR-17-5p	BMP-2	(Li *et al.*, 2013)[68]
miR-106a	BMP-2	(Li *et al.*, 2013)[68]
miR-125b	OSX	(Chen *et al.*, 2004)[107]
miR-637	OSX	(Zhang *et al.*, 2011)[108]
miR-188	HDAC9	(Li *et al.*, 2015)[114]
miR-141-3p	Wnt	(Qiu and Kassem, 2014)[120]
miR-338-3P	RUNX2 and Fgfr2	(Liu *et al.*, 2014; Guo*et al.*, 2015)[106]

CONCLUSION AND FUTURE PERSPECTIVES

miRNAs possess various features such as easy accessibility, tissue specificity and sensitivity. They usually target osteogenic specific genes and leads to bone formation. This is well applicable in various bone ailments. Nonetheless, the mechanism still remain ambiguous. miRNAs play significant roles in the induction of MSCs into osteoblasts. miRNAs obstruct the negative regulators of signaling pathways operating in the cells throughout the process of osteoblast differentiation. They involve both directly and indirectly in phenotype development by both supporting and suppressing positive and negative transcription factors in signalling pathways. This points towards a regulatory role which mRNAs can play in osteoblast differentiation.

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REFERENCES

[1] IHGSC. Finishing the euchromatic sequence of the human genome. Nature 2004; 431: 931-45.
[2] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A et al . Landscape of transcription in human cells. Nature 2012; 89: 101-8.
[3] Mercer TR, Dinger ME &Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009; 10: 155-9.
[4] Lee RC, Feinbaum RL, Ambros V. The C. elegansheterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75:843-854.
[5] Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 1993; 75:855-862.
[6] Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditiselegans. Nature 2000; 403:901-906.
[7] Basyuk E, Suavet F, Doglio A, Bordonne R, Bertrand E. Human let-7 stem-loop precursors harbor features of RNase III cleavage products. Nucleic Acids Res 2003; 31:6593-6597.
[8] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116:281-297.
[9] Han J. The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev 2004; 18: 3016-3027.
[10] Han J.Molecular basis for the recognition of primary microRNAs by the DroshaDGCR8 complex. Cell 2006; 125: 887-901.
[11] MacraeIJ.Structural basis for double-stranded RNA processing by Dicer. Science 2006; 311: 195-198.
[12] Bernardo BC, Charchar FJ, Lin RC, McMullen JR. A microRNA guide for clinicians and basic scientists: background and experimental techniques. Heart Lung Circ 2012; 21:131-142.
[13] Berezikov E, Chung WJ, Willis J, Cuppen E, Lai EC. Mammalian mirtron genes. Mol Cell 2007; 28: 328-336.
[14] Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell 2007; 130: 89-100.
[15] Peel, N. Bone remodelling and disorders of bone metabolism. Surgery 2009; 27(2):70-74.
[16] Mulari MT, Qu Q, Harkonen PL. &Vaananen HK. Osteoblast-like cells complete osteoclastic bone resorption and form new mineralized bone matrix in vitro. Calcified Tissue International 2004; 75(3):253-261.
[17] Neve A, Corrado A. &Cantatore FP. Osteoblast physiology in normal and pathological conditions. Cell and Tissue Research2011; 343(2): 289-302.
[18] Oishi Y, Watanabe Y, Shinoda S, Naka M, Ozawa Y. & Matsuyama T. The IL6 gene polymorphism − IL17F gene polymorphism influence bone mineral density in young and elderly Japanese women. Gene 2012; 504(1): 75-83.
[19] Ralston SH. Structure and metabolism of bone. Medicine 2005; 33(12): 58-60.
[20] Kazama JJ, Koda R, Yamamoto S, Narita I, Gejyo F. &Tokumoto A. Cancellous bone volume is an indicator for trabecular bone connectivity in dialysis patients. Clinical Journal of the American Society of Nephrology 2010; 5(2):292-298.
[21] Crockett JC, Rogers MJ, Coxon FP, Hocking LJ &Helfrich MH. Bone remodelling at a glance. Journal of Cell Science2011; 124(7): 991-998.

[22] Sims NA. & Martin TJ. Coupling the activities of bone formation and resorption: A multitude of signals within the basic multicellular unit. Bone Key Reports2014; 3: 1-10.
[23] Rucci N. Molecular biology of bone remodelling. Clinical Cases in Mineral and Bone Metabolism 2008; 5(1): 49.
[24] Shahnazari M, Chu V, Wronski TJ, Nissenson RA. & Halloran BP. CXCL12/CXCR4 signaling in the osteoblast regulates the mesenchymal stem cell and osteoclast lineage populations. FASEB Journal2013; 27(9): 3505-3513.
[25] Bellido T, Plotkin LI. &BruzzanitiA.Chapter 2 - Bone cells. In: Burr, D. B. and Allen, M. R. (eds.), Basic and applied bone biology 2014. San Diego: Academic Press, pp 27-45.
[26] Hadjidakis DJ. &Androulakis II. Bone remodeling. Annals of the NewYork Academy of Sciences 2006; 1092(1):385-396.
[27] Downey PA. & Siegel MI. Bone biology and the clinical implications for osteoporosis. Physical therapy 2006; 86(1): 77-91.
[28] Andrades JA, Narvaez-Ledesma L, Ceron-Torres L, Cruz-Amaya AP, Lopez- Guillen D, Mesa-Almagro ML. & Moreno-Moreno JA. Bone engineering: A matter of cells, growth factors and biomaterials. In, Regenerative Medicine and Tissue Engineering 2013.
[29] Tanaka Y, Nakayamada S. & Okada, Y. Osteoblasts and osteoclasts in bone remodeling and inflammation. Current Drug Targets - Inflammation & Allergy 2005;4(3): 325-8.
[30] Caetano-Lopes J, Canhao H. & Fonseca JE. Osteoblasts and bone formation. ActaReumatologica Portuguesa 200; 32(2): 103-110.
[31] Kini U. &Nandeesh B. (2012). Physiology of bone formation, remodeling and metabolism. Radionuclide and Hybrid Bone Imaging: Springer, pp 29-57.
[32] Neve A, Corrado A. &Cantatore FP. Osteoblast physiology in normal and pathological conditions. Cell and Tissue Research2011; 343(2): 289-302.
[33] Harimoto K, Yoshida Y, Yoshihara K, Nagaoka N, Matsumoto T. &Tagawa Y. Osteoblast compatibility of materials depends on serum protein absorbability in osteogenesis. Dental materials journal 2012; 31(4): 674-680.
[34] Calabrese G, Bennett BJ, Orozco L, Kang HM, Eskin E, Dombret C, De Backer O, Lusis AJ. & Farber CR Systems genetic analysis of osteoblast-lineage cells. PLoS genetics 2012; 8(12): e1003150.
[35] Link DC. Osteocytes link bone maintenance to blood homeostasis. Blood 2013;121(6): 867-868.
[36] Dallas SL. &Bonewald LF. Dynamics of the transition from osteoblast to osteocyte. Annals of the New York Academy of Sciences 2010; 1192(1): 437-443.
[37] Xia X, Batra N, Shi Q, Bonewald LF, Sprague E. & Jiang JX. Prostaglandin promotion of osteocyte gap junction function through transcriptional regulation of connexin 43 by glycogen synthase kinase 3/beta-catenin signaling. Molecular Cell Biology2010; 30(1): 206-219.
[38] Nakamura, H. Morphology, function and differentiation of bone cells.Journal of Hard Tissue Biology 2007; 16(1):15-22.
[39] Long, F. Building strong bones: Molecular regulation of the osteoblast lineage. Nature Reviews Molecular Cell Biology 2012; 13(1): 27-38.
[40] Akiyama H, Kim JE, Nakashima K, Balmes G, Iwai N, Deng JM, Zhang Z, Martin JF, Behringer RR, Nakamura T. & de Crombrugghe B. Osteochondroprogenitor cells are derived from Sox9 expressing precursors. Proceedings of the National Academy of Sciences of the United States of America 2005; 102(41): 14665-14670.
[41] Robling AG, Castillo AB, Turner CH. Biomechanical and molecular regulation of bone remodeling. Annual review of biomedical engineering 2006; 8: 455-498.
[42] Nishio Y, Dong Y, Paris M, O'Keefe RJ, Schwarz EM. &Drissi H. Runx2-mediated regulation of the zinc finger Osterix/Sp7 gene. *Gene* 2006; 372:62-70.
[43] Feldman D. (2013). Osteoporosis. 4th ed. Chapter 6 Osteoblast biology, *Elsevier Science*, 93-130.
[44] Clarke B. Normal bone anatomy and physiology. Clinical Journal of the American Society of Nephrology2008; 3(3): 131-139.
[45] Herman S, Kronke G, Schett G. Molecular mechanisms of inflammatory bone damage: emerging targets for therapy. Trends in molecular medicine2008; 14(6): 245-253.
[46] Gong M, Ma J, Guillemette R, Zhou M, Yang Y. &Yang Y. miR-335 inhibits small cell lung cancer bone metastases via IGF-IR and RANKL pathways. Mol Cancer Res 2014; 12(1):101-10.
[47] Shi C, Qi J, Huang P et al. MicroRNA-17/20a inhibits glucocorticoid-induced osteoclast differentiation and function through targeting RANKL expression in osteoblast cells. Bone 2014; 68:67–75.

[48] Wang T, Yin H, Wang J, Li Z, Wei H, Liu Z et al. MicroRNA-106b inhibits osteoclastogenesis and osteolysis by targeting RANKL in giant cell tumor of bone. Oncotarget 2015; 6(22): 18980–18996.
[49] Mizoguchi F, Izu Y, Hayata T, Hemmi H, Nakashima K, Nakamura T, S. Osteoclast-specific Dicer gene deficiency suppresses osteoclastic bone resorption, J. Cell Biochem 2010; 109:866–875.
[50] Blüml S, Bonelli M, Niederreiter B, Puchner A, Mayr G, Hayer S. Essential role of microRNA-155 in the pathogenesis of autoimmune arthritis in mice, Arthritis Rheum 2011; 63:1281–1288.
[51] Zhao JJ, Wu ZF, Wang L, Feng DH, Cheng L. MicroRNA-145 Mediates Steroid-Induced Necrosis of the Femoral Head by Targeting the OPG/RANK/RANKL Signaling Pathway. PLoS ONE 2016; 11(7):1-15.
[52] Chen C; Cheng P, Xie H, Zhou HD, Wu XP, Liao E. miR-503 regulates osteoclastogenesis via targeting rank. J. Bone Miner. Res 2014; 29 :338–347.
[53] Hwang S, Park SK, Lee HY et al. miR-140-5p suppresses BMP2-mediated osteogenesis in undifferentiated human mesenchymal stem cells. FEBS Lett 2014; 588(17): 2957–2963.
[54] Kureel J, Dixit M, Tyagi AM, Mansoori MN, Srivastava K et al. miR-542-3p suppresses osteoblast cell proliferation and differentiation, targets BMP-7 signaling and inhibits bone formation. Cell Death Dis 2014; 5(2): e1050.
[55] Goettsch, C. *et al.* miR-125b regulates calcification of vascular smooth muscle cells. Am. J. Pathol2011; 179: 1594–1600.
[56] Li Z. *et al.* A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc. Natl Acad. Sci. USA2008; 105: 13906–13911.
[57] Schaap-Oziemlak AM. *et al.* MicroRNA hsa-miR-135b regulates mineralization in osteogenic differentiation of human unrestricted somatic stem cells. Stem Cells Dev2010; 19; 877–885.
[58] Itoh T, Nozawa Y. &Akao Y. MicroRNA-141 and -200a are involved in bone morphogenetic protein-2-induced mouse pre-osteoblast differentiation by targeting distal-less homeobox 5. J. Biol. Chem 2009;284:19272–19279.
[59] Itoh T, Takeda S. &Akao Y. MicroRNA-208 modulates BMP-2-stimulated mouse preosteoblast differentiation by directly targeting V-etserythroblastosis virus E26 oncogene homolog 1. J. Biol. Chem2010*;* 285: 27745–27752.
[60] Zhang JF. *et al.* MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. RNA Biol 2011;8: 16043.
[61] Mizuno Y, Tokuzawa Y, Ninomiya K, Yagi Y, Yatsuka-Kanesaki T, Suda. miR-210 promotes osteoblastic differentiation through inhibition of AcvR1b, FEBS Lett 2009;583:2263–2368.
[62] Luzi E, Marini F, Sala SC, Tognarini I, Galli G, Brandi ML. Osteogenic differentiation of human adipose tissue derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor. J Bone Miner Res 2008; 23(2): 287–295.
[63] Li Z, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM. A microRNA signature for a BMP2-induced osteoblast lineage commitment program, Proc. Natl. Acad. Sci. USA2008; 105:13906–13911.
[64] Li H, Xie H, Liu W, et al., A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans, J. Clin.Invest2009;119:12:3666–3677.
[65] Sun Q, Mao S, Li H, Zen K, Zhang CY, Li L. Role of miR-17 family in the negative feedback loop of bone morphogenetic protein signaling in neuron, PLoS One 2013; 8: e83067.
[66] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets Cell 2005; 120:15–20.
[67] Hu R, Liu W, Li H, Yang L, Chen C, Xia ZY. A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation, J. Biol. Chem 2011; 286: 12328–12339.
[68] Li H, Li T, Wang S, et al. miR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res 2013; 10(3):313–324.
[69] Fang T, Wu Q, Zhou L, Mu S, Fu Q. miR-106b-5p and miR-17-5p suppress osteogenic differentiation by targeting Smad5 and inhibit bone formation, Exp Cell Res 2016;347: 74–82.
[70] Shi K, Lu J, Zhao Y, Wang L, Li J, Qi B. MicroRNA-214 suppresses osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. Bone 2013;55: 487–494.
[71] Yang L, Cheng P, Chen C, He HB, Xie GQ, Zhou HD. MiR-93/Sp7 function loop mediates osteoblast mineralization. J. Bone Miner. Res 2012; 27:1598–1606.
[72] Wu T, Zhou H, Hong Y, Li J, Jiang X, Huang H. miR-30 family members negatively regulate osteoblast differentiation. J Biol Chem. 2012; 287(10):7503–11.
[73] Zeng Y, Qu X, Li H, Huang S, Wang S, Xu Q. MicroRNA-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting BMPR2. FEBS Lett 2012; 586:2375–2381.

[74] Wagner DO, Sieber C, Bhushan R, Borgermann JH, Graf D, Knaus P. BMPs: from bone to body morphogenetic proteins. Sci Signal 2010; 3:mr1.
[75] Yi JJ, Barnes AP, Hand R, Polleux F, Ehlers MD. TGF-beta signaling specifies axons during brain development. Cell 2010; 142:144-57.
[76] Mayorga ME, Penn MS. miR- 145 is differentially regulated by TGF- b1 and ischaemia and targets disabled- 2 expression and wnt/b- catenin activity. J. Cell. Mol. Med2012; 16 (5): 1106–1113.
[77] Li Z. *et al.* Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. J. Biol. Chem2009; 284: 15676–15684.
[78] Williams BO and Insogna KL. "Where Wnts went: the exploding field of Lrp5 and Lrp6 signaling in bone," Journal of Bone and Mineral Research 2009; 24(2):171–178.
[79] Gong Y, Slee RB, Fukai N et al. "LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development," Cell 2001; 107(4): 513–523.
[80] Whyte MP, Reinus WH, and Mumm S, "High-bone-mass disease and LRP5," The New England Journal of Medicine 2004; 350(20): 2096–2096.
[81] Jin T, Fantus IG, and Sun J. "Wnt and beyond Wnt: multiple mechanisms control the transcriptional property of β-catenin,"Cellular Signalling 2008; 20(10):1697–1704.
[82] Sun K, Wang J, Liu F, Ji Z, Guo Z, Zhang C. Ossotide promotes cell differentiation of human osteoblasts from osteogenesisimperfecta patients by up-regulating miR-145.Biomedicine & Pharmacotherapy 2016;83: 1105–1110.
[83] Wang T and Xu Z: Mir-27 promotes osteoblast differentiation by modulating Wntsignaling. BiochemBiophys Res Commun 2010; 402: 186-189.
[84] Kapinas K, Kessler CB. & Delany AM. miR-29 suppression of osteonectin in osteoblasts: regulation during differentiation and by canonical Wntsignaling. J. Cell. Biochem2009.108, 216–224.
[85] Kapinas, K, Kessler C, Ricks T, Gronowicz G. & Delany AM. miR-29 modulates Wntsignaling in human osteoblasts through a positive feedback loop. J. Biol. Chem2010; 285: 25221–25231.
[86] Zhang J. *et al.* Effects of miR-335-5p in modulating osteogenic differentiation by specifically down-regulating Wnt antagonist DKK1. J. Bone Miner. Res 2011;26: 1953–1963.
[87] Osta B, Roux JP, Lavocat F, Pierre M, Ndongo-Thiam N, Boivin G.Differential effects of IL-17A and TNF-a on osteoblastic differentiation of isolated synoviocytes and on bone explants from arthritis patients. Frontiers in immunology 2015; 6:1-8.
[88] Yang N, Wang G, Hu C, Shi Y, Liao L, Shi S. Tumor Necrosis Factor a Suppresses the Mesenchymal Stem Cell Osteogenesis Promoter miR-21 in Estrogen Deficiency–Induced Osteoporosis. Journal of Bone and Mineral Research 2013; 28(3): 559–573.
[89] Fiuza UM. and Arias AM. Cell and molecular biology of Notch. J. Endocrinol 2007; 194: 459-474.
[90] Engin F, Yao Z, Yang T, Zhou G, Bertin T, Jiang MM.Dimorphic effects of Notch signaling in bone homeostasis. Nat. Med 2008; 14: 299-305.
[91] Shimizu T, Tanaka T, Iso T, Matsui H, Ooyama Y, Kawai-Kowase K. Notch signaling pathway enhances bone morphogenetic protein 2 (BMP2) responsiveness of Msx2 gene to induce osteogenic differentiation and mineralization of vascular smooth muscle cells. J BiolChem 2011; 286: 19138-19148.
[92] Shindo K, Kawashima N, Sakamoto K, Yamaguchi A, Umezawa A, Takagi M. Osteogenic differentiation of the mesenchymal progenitor cells, Kusa is suppressed by Notch signaling. Exp Cell Res 2003; 290: 370-380.
[93] Ugarte F, Ryser M, Thieme S, Fierro FA, Navratiel K, Bornhäuser M. Notch signaling enhances osteogenic differentiation while inhibiting adipogenesis in primary human bone marrow stromal cells. ExpHematol 2009; 37: 867-875.
[94] Sun F, Wan M, Xu X, Gao B, Zhou Y, Sun J. Crosstalk between miR-34a and notch signaling promotes differentiation in apical papilla stem cells (SCAPs). J Dent Res 2014; 93: 589-595.
[95] Li J, Dong J, Zhang ZH, Zhang DC, You XY, Zhong Y. Mir-10a restores human mesenchymal stem cell differentiation by repressing KLF4. J Cell Physiol 2013; 228: 2324-2336.
[96] Gamez B, Rodriguez-Carballo E, Bartrons R, Rosa JL and Ventura F: microRNA-322 (miR-322) and its target protein Tob2 modulate osterix (osx) mrna stability. J BiolChem 2013; 288: 14264-14275.
[97] Hassan, M. Q. *et al.* A network connecting Runx2, SATB2, and the miR-23a~27a~24–2 cluster regulates the osteoblast differentiation program. Proc. Natl Acad. Sci. USA 2010;107:19879–19884.
[98] Huang J, Zhao L, Xing L, Chen D: MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell diff erentiation. Stem Cells2010; 28:357-364.

[99] Tomé M, López-Romero P, Albo C, Sepúlveda JC, Fernández-Gutiérrez B, Dopazo A, et al. miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells. Cell Death Differ 2011; 18(6): 985–995.
[100] Ko, JY, Chuang PC, Ke HJ, Chen YS, Sun YC, and Wang FS. "MicroRNA-29a mitigates glucocorticoid induction of bone loss and fatty marrow by rescuing Runx2 acetylation,"Bone 2015; 81:80–88.
[101] Vimalraj S, Partridge NC, and Selvamurugan N. "A positive role of microRNA-15b on regulation of osteoblast differentiation,"Journal of Cellular Physiology2014; 229(9): 1236– 1244.
[102] Li H, Xie H, Liu Wet al., "A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans,"Journal ofClinical Investigation 2009; 119(12):3666–3677.
[103] Xia ZY, Hu Y, Xie PL et al., "Runx2/miR-3960/miR-2861 positive feedback loop is responsible for osteogenictransdifferentiation of vascular smooth muscle cells,"BioMed Research International 2015.;45;105-117.
[104] Song MY, Yu JZ, Zhao DM et al. "The time-dependent manner of sinusoidal electromagnetic fields on rat bone marrow mesenchymal stem cells proliferation, differentiation, and mineralization,"Cell Biochemistry and Biophysics 2014; 69(1): 47–54.
[105] Liao L, Yang X, Su X et al. "Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow,"Cell Death and Disease 2013;4(4):e600.
[106] Liu H, Sun Q, Wan C, Li L, Zhang L, and Chen Z. "MicroRNA-338-3p regulates osteogenic differentiation of mouse bone marrow stromal stemcells by targeting Runx2 and Fgfr2,"Journal of Cellular Physiology 2014; 229(10): 1494–1502.
[107] Chen S, Yang L, Jie Q et al. "MicroRNA-125b suppresses the proliferation and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells,"Molecular Medicine Reports 2014; 9(5): 1820–1826.
[108] Zhang JF, Fu WM, He ML et al. "MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix,"Molecular Biology of the Cell 2011; 22(21): 3955–3961.
[109] Jia, J, Tian, Q, Ling, S, Liu, Y, Yang, S. and Shao, Z. MiR-145 suppresses osteogenic differentiation by targeting Sp7. FEBS Lett 2013; 587:3027–3031.
[110] Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR. and de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002; 108:17–29.
[111] Fukuda T, Ochi H, Sunamura S, Haiden A, Bando W, Inose H, Okawa A, Asou Y and Takeda S: MicroRNA-145 regulates osteoblastic differentiation by targeting the transcription factor Cbfb. FEBS Lett 2015; 589: 3302-3308.
[112] Sun K, Wang J, Liu F, Ji Z, Guo Z, Zhang C,Yao M. Ossotide promotes cell differentiation of human osteoblasts from osteogenesisimperfecta patients by up-regulating miR-145.Biomedicine & Pharmacotherapy 2016;83: 1105–1110.
[113] Liu X, Cai F, Liu L, Zhang Y. & Yang A. MicroRNA- 210 is involved in the regulation of postmenopausal osteoporosis through promotion of VEGF expression and osteoblast differentiation. Biological Chemistry 2015; 396(4): 339– 347.
[114] Li H, Li T, Fan J, Li T, Fan L, Wang S, Weng X, Han Q. & Zhao RC. miR-216a rescues dexamethasone suppression of osteogenesis, promotes osteoblast differentiation and enhances bone formation, by regulating c-Cbl-mediated PI3K/AKT pathway. Cell Death and Differentiation2015b; 22(12): 1935–1945.
[115] Ko J, Chuang P, Ke H, Chen Y, Sun Y. and Wang F. MicroRNA-29a mitigates glucocorticoid induction of bone loss and fatty marrow by rescuing Runx2 acetylation. Bone 2015; 81: 80-88.
[116] Zhang Z, Wippo CJ, Wal M, Ward E, Korber P. & Pugh BF. A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. Science 2011; 332(6032); 977-80.
[117] You L, Gu W, Chen L, Pan L, Chen J. &Peng Y. MiR-378 overexpression attenuates high glucose-suppressed osteogenic differentiation through targeting CASP3 and activating PI3k/Aktsignaling pathway. International Journal of Clinical and Experimental Pathology 2014; 7(10):7249– 7261.
[118] Zuo B, Zhu J, Li J, Wang C, Zhao X, Cai G, Li Z, Peng J, Wang P, Shen C, Huang Y, Xu J, Zhang X. & Chen X.. MicroRNA-103a functions as a mechanosensitive microRNA to inhibit bone formation through targeting Runx2. Journal of Bone and Mineral Research 2015; 30 (2): 330–345.
[119] 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. Human Molecular Genetics 2012; 21(13): 2991–3000.

[120] Qiu W &Kassem M. MiR-141-3p inhibits human stromal (mesenchymal) stem cell proliferation and differentiation. BiochimicaetBiophysicaActa-Molecular Cell Research 2014; 1843(9); 2114–2121.