miRNAs in Bone Development

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Abstract: Skeletal development is a multistage process during which mesenchymal progenitor cells undergo proliferation and differentiation and subsequently give rise to bone and cartilage forming cells. Each step is regulated by various transcription factors and signaling molecules. microRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression. Several in vivo and in vitro studies have shown that miRNAs play significant roles in skeletal development. Identifying their functions may give insights into the treatment of developmental disorders of the skeleton. This review summarizes miRNAs that have been shown to participate in various stages of skeletal development by targeting crucial factors.

Keywords: Bone, Cartilage, Chondrocytes, Mesenchymal stem cells, mi-RNAs, Osteoblasts, Skeletal development.

INTRODUCTION

The vertebrate skeleton is composed of cartilage and bone and is derived from three mesodermal lineages. During embryogenesis, cranial neural crest cells form the craniofacial skeleton, paraxial mesodermal cells give rise to the axial skeleton, and lateral plate mesodermal cells form the appendicular skeleton. Migration of these cells to the location of future bone development leads to the formation of mesenchymal condensations. There are several genes that are essential for early patterning of the axial skeleton. Among them, the Hox cluster genes that are expressed in different combinations at particular skeletal axis levels, determine the position of the future vertebrae [1-3].

In locations where endochondral ossification will take place, cells within the mesenchymal condensations first differentiate into chondrocytes, producing a cartilage template (anlage). During endochondral ossification, cartilage is replaced by mineralized bone and bone marrow. Several bones such as the craniofacial bones and the clavicle are formed through direct differentiation of cells in mesenchymal condensations into osteoblasts; this process is called intramembranous ossification. However, the majority of bones are formed by endochondral ossification [4].

Longitudinal bone growth is driven by the growth plate cartilage, where chondrocyte proliferation and differentiation are tightly coordinated. The growth plate consists of three groups of chondrocytes. Slowly proliferating, resting/perichondrial chondrocytes differentiate into proliferating chondrocytes that further differentiate into postmitotic hypertrophic chondrocytes. The latter are replaced by mineralized bone [5]. Signaling molecules such as Indian hedgehog (Ihh), parathyroid hormone-related peptide (PTHrP), bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) play important roles in the regulation of chondrocyte differentiation. Ihh, expressed by prehypertrophic and hypertrophic chondrocytes, regulates chondrocyte proliferation and differentiation, and expression of PTHrP in resting/periarticular chondrocytes. PTHrP negatively regulates differentiation of prehypertrophic and hypertrophic chondrocytes creating an autoregulatory system. In addition, Ihh couples osteogenesis and chondrogenesis by inducing osteoblast differentiation of perichondrial cells [6]. BMP signaling stimulates differentiation of prechondrogenic cells into chondrocytes and differentiation of proliferating chondrocytes into hypertrophic chondrocytes [7]. FGFs are a large family of secreted proteins with a vast array of biological functions during bone development, including regulation of chondrocyte differentiation and bone formation. They bind to cell surface tyrosine kinase receptors (fibroblast growth factor receptors, FGFRs 1-4), activating multiple signaling pathways such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and Janus kinase/signal transducer and activator of transcription (JAK/STAT1) [8]. Additionally, transcription factors such as sex determining region Y (SRY) box 9 (Sox9), myocyte enhancer factor 2 (MEF2) family, histone deacetylase 4 (Hdac4) and runt related transcription factor 2 (Runx2) are crucial for chondrocyte differentiation [5]. Sox9 is expressed in osteo-chondro-progenitor cells and upon cell differentiation it is expressed in chondrocytes. It regulates the expression of collagens type II, XI and aggrecan (Acan), being important for chondrocyte function [9]. Runx2 is expressed in mesenchymal condensations, perichondrial cells, hypertrophic chondrocytes and osteoblasts, and one of its functions is to regulate chondrocyte hypertrophy [10-12]. After chondrocytes undergo terminal differentiation (hypertrophy), they mineralize and are replaced by bone. Osteoblasts, the cells that produce mineralized bone matrix, are differentiated from...
local mesenchymal progenitor cells. Ihh, wingless-type MMTV integration site family (Wnt) and Notch signaling pathways as well as Runx2 and Osx (Sp7) transcription factors regulate osteoblast differentiation [13]. Wnt signaling plays an essential role in the regulation of bone mass. In the canonical Wnt signaling pathway, Wnt stabilizes β-catenin, promoting its nuclear localization. Subsequently, β-catenin directly regulates gene expression by forming a transcriptional complex with lymphoid enhancer-binding factor 1 (LEF-1) [14]. Notch signaling, through its ligands (Jagged 1,2,Delta-like 1,3,4) and receptors (Notch1-4), suppresses the differentiation of mesenchymal precursors into preosteoblasts [15, 16]. Osx (Sp7) is a zinc finger transcription factor, required for osteoblast differentiation. Osx deficient mice show an absence of osteoblasts and bone formation [17]. The above transcription factors and signaling molecules are essential for the regulation of skeletogenesis. Changes in their expression can significantly influence skeletal development.

Among factors that can alter gene expression are microRNAs (miRNAs). They are single stranded non-coding RNAs, ~22 nucleotides long that regulate gene expression at the post-transcriptional level. miRNAs are generated after multistep processing of their precursors. Initially, long primary transcripts (pri-miRNAs) are processed into small hairpin RNAs (pre-miRNAs) by the microprocessor complex comprised of Drosha and DGCR8. Pre-miRNAs are further processed by Dicer into their mature form. miRNAs bind mainly to the 3’ UTR (untranslated region) of target mRNAs through partial base complementarity. This binding most often results in suppression of translation and mRNA degradation [18]. miRNAs have important roles in diverse cellular functions, such as metabolism, differentiation and apoptosis. Their role in skeletal development has been demonstrated by several studies.

This review will summarize studies on miRNAs that play a role in various stages of skeletal development in vivo and those that have been shown to be related to critical factors for skeletal development in vitro.

**miRNAs in Early Skeletal Development**

The overall importance of miRNAs during early skeletal development was first demonstrated by genetic ablation of Dicer.

In mice, the Dicer gene was conditionally deleted in the limb mesenchyme [19] and in chondrocytes [20] causing a global reduction of miRNAs in these tissues. The Cre/loxP recombination system is an effective tool for generating tissue specific-targeted mutants. The enzyme Cre recombinase catalyzes the rearrangement of DNA fragments flanked by two 34bp loxP sites (floxed) [21]. In the limb mesenchyme, floxed Dicer was deleted using a transgene expressing Cre recombinase under the control of a Prxl driven enhancer, expressed in the early limb bud mesenchyme and whose activity starts around embryonic day E9.5. Dicer deletion in developing limbs caused a significant reduction of the limb size with increased cell death of limb bud cells. It resulted in morphological malformations such as twisted long bones of arms and legs, and delayed development from cartilage to bone. However, postnatally, all of the differentiated cell types expected in the mature limb bud were present in the Dicer deficient limbs. This suggests that Dicer is required for morphogenesis but not for patterning of the developing limbs [19]. Deletion of Dicer in chondrocytes resulted in significant growth defects and early death by the time of weaning [20]. These mutant mice showed a proportional reduction in skeletal size. The growth defect of the skull and maxilla caused a relative overgrowth of the mandible and lower incisors. A dramatic decrease in proliferation of growth plate chondrocytes, and an increased differentiation of proliferating chondrocytes towards postmitotic hypertrophic chondrocytes were observed [20]. Similar to the Dicer knockout (KO) mice, deletion of Drosha or DGCR8 in chondrocytes caused overall reduction of miRNAs, leading to reduction in proliferating chondrocytes of the growth plate, reduced longitudinal growth of the skull and impaired skeletal development. However, these mice died perinatally [22]. These studies show that miRNAs are necessary for the growth of skeletal tissues during embryogenesis.

**miR-196, one of the few miRNAs that are expressed within the Hox clusters, has been shown to target multiple Hox genes, which makes it potentially important for early development [23].** When miR-196 was knocked-down by local antagonir injection in the presomitotic mesoderm of chicken stage 11 embryos, *HoxB8* expression was expanded anteriorly and the last cervical vertebra was transformed toward a thoracic identity [24]. In many embryos, this was accompanied by the presence of an ectopic rib. Also, when miR-196 was systemically knocked-down, vertebral malformations such as split vertebrae and vertebral fusions were observed. Somites are blocks of mesoderm in the developing vertebrate embryo that give rise to important structures such as skeletal muscle, cartilage, tendons, vertebrae. When miR-196 was overexpressed in zebrafish embryos, it reduced total somite number and caused axial abnormalities [25]. miR-196 is an example of a specific miRNA whose deletion leads to abnormalities in early skeleton.

**miRNAs in Postnatal Skeletal Development**

Important in vivo studies have revealed a significant role for miRNAs in growth plate maturation (miR-140 and let-7) and in osteoblast function (miR-2861, miR-3960, miR-182, miR-199, miR-214, miR-17-92 and miR-34) (Table 1).

**Role in Growth Plate Maturation**

The chondrocyte specific miRNA, miR-140, is highly expressed in the growth plate. miR-140 deletion in mice causes a mild reduction in the length of endochondral bones and in longitudinal growth of the skull [26, 27]. Loss of miR-140 led to premature chondrocyte differentiation towards hypertrophic chondrocytes, while the differentiation of resting chondrocytes to proliferating chondrocytes was delayed. The transcription factor Sox9 was found to regulate miR-140 expression in studies using the chondrocyte cell line, ATDC5 [28], in zebrafish [29] and in mice [30]. miR-140 interacts with the PTHrP/Hdac4 pathway to control chondrocyte differentiation. Deletion of one allele of PTHrP or Hdac4 caused growth impairment in mice null for miR-140. MEF2 transcription factors directly stimulate hypertrophic
miRNAs in Bone Development

Table 1. miRNAs regulating skeletal development, studied in vivo.

| miRNA        | Biological Function                                                                 | Target Gene                        | Model                                                                 |
|--------------|--------------------------------------------------------------------------------------|------------------------------------|----------------------------------------------------------------------|
| All miRNAs   | Limb morphogenesis                                                                   | several                            | Global deletion of miRNAs in vivo through Dicer deletion in:         |
|              | Regulation of growth plate chondrocytes proliferation and differentiation             |                                    | mouse limb mesenchyme [19]                                            |
|              | Differentiation of osteoblast progenitors, suppression of mature osteoblasts          |                                    | mouse Cartilage [20]                                                  |
| miR-196      | Development of vertebrae and axial skeleton                                          | Multiple *Hox* genes                | mouse osteoblasts [33-34]                                             |
| miR-140      | Role in growth plate chondrocyte differentiation                                       | Regulates PTHrP/Hdac4/Mef2c pathway | Mice [31]                                                             |
| Let-7        | Role in growth plate chondrocyte proliferation                                        | *E2F5, Cdc34*                       | Mice [32]                                                             |
| miR-2861     | Increases osteoblast differentiation and bone mass                                     | *Hdac5*                            | Mice [35]                                                             |
|              |                                                                                      |                                    | Human mutation [35]                                                   |
| miR-3960     | Increases osteoblast differentiation and bone mass                                     | *Hoxa2*                            | Mice [36]                                                             |
| miR-182      | Inhibits osteoblast proliferation and differentiation                                 | *Foxo1*                            | Zebrafish [37]                                                        |
| miR-199 and miR-214 | Regulate skeletal growth and bone mass                                           |                                    | Mice [39]                                                             |
| miR-214      | Its overexpression inhibits bone formation                                            | *Atf4*                             | Mice [40]                                                             |
| miR-17-92    | Regulates osteoblast proliferation, differentiation and skull and digit development    |                                    | Human mutations [42-45], mice [41, 46]                                |
| miR-34a      | miR-34a                                                                              | *Cnd1, Cdk4, Cdk6, E2F3, Cdc25a*   | *In vitro* in human MSCs and in mice [48]                            |
| miR-34b/miR-34c | miR-34b/miR-34c                                                                     | *Cnd1, Cdk4, Cdk6, Satb2*          | Mice [47]                                                             |
| miR-188      | Increases adipocyte differentiation and reduces osteoblast differentiation            | *Hdac9, Rictor*                     | Human BMSCs and mice [51]                                             |

chondrocyte differentiation. MEF2 function is inhibited by Hdac4, the activity of which, is enhanced by PTHrP. Upon miR-140 deletion, there was an increase in Mef2c expression, with normal Hdac4 levels, showing that miR-140 interacts with the PTHrP/Hdac4 pathway at the level of Mef2c [31].

Growth plate chondrocyte proliferation was found to be regulated by let-7 miRNAs. In mice, overexpression of Lin28a, a let-7 inhibitor, resulted in mild growth impairment due to a reduction in chondrocyte proliferation [32]. The cell cycle regulators cell division cycle 34 (*Cdc34*) and E2F transcription factor 5 (*E2F5*) are let-7 targets that mediate this effect. Whereas deficiency of let-7 or miR-140 miRNAs alone caused a mild skeletal phenotype, their combination had a synergistic effect leading to dramatic growth impairment [32]. Thus, let-7 and miR-140 regulate chondrocyte proliferation and differentiation respectively, coordinating skeletal development.

Role in Osteoblasts

*Dicer* was deleted in osteoblast progenitors using Osx-Cre transgenic mice, in which, Cre recombinase is driven by Osx promoter. Dicer loss in these cells caused a reduction in mature osteoblasts and altered texture of mineralized bone matrix without a reduction in bone volume at 4-6 weeks of age [33]. On the contrary, Dicer deletion in mature osteoblasts and osteocytes using osteocalcin-Cre transgenic mice led to increases in bone mass and in expression of the osteoblast markers type I collagen (*Coll1a1*), Osx (*Sp7*), and osteocalcin (*bglap*) [34]. These studies suggest that miRNAs, overall, promote the differentiation of osteoblast progenitors but suppress the functions of mature osteoblasts.

Regarding specific miRNAs, miR-2861 is expressed mainly in osteoblasts and targets histone deacetylase 5 (*Hdac5*), an enzyme that regulates various cellular proteins, including Runx2 and Mef2 transcription factors [35]. miR-
miRNA profiling in differentiated mouse calvarial osteoblasts, showed that miR-34b and miR-34c were significantly upregulated (~1.8 fold) and had relatively restricted expression in osteoblasts [47]. Transgenic mice overexpressing miR-34c had a low bone mass phenotype at 3 months of age. The number of osteoblasts and bone formation rate were reduced. Mice in which both miR-34b and miR-34c were deleted in mature osteoblasts using Col1Cre transgenic mice showed increases in bone mass, osteoblast proliferation and bone formation rate. Cell cycle regulators, including cyclin D1 (Cdcn1) and cyclin dependent kinases Cdk4 and Cdk6 were identified as targets of miR-34b and c. Additionally, miR-34b/c deletion in osteoblasts increased osteoblast differentiation in vivo and ex vivo. Sath2 (special AT-rich sequence binding protein 2), a protein that promotes osteoblast differentiation, was found to be the target mediating this effect. Thus, miR34b/c regulate skeletogenesis by inhibiting osteoblast proliferation and terminal differentiation. [47]. On the other hand, an ex vivo study, demonstrated that miR-34a was significantly upregulated upon differentiation of human mesenchymal stem cells (MSCs) into osteoblasts. miR-34b showed a smaller increase but miR-34c was not detectable [48]. miR-34a overexpression in human MSCs inhibited osteoblast differentiation and mineralization. miR-34a targeted Jag1, encoding for a Notch ligand. Also, miR-34a targeted the cell cycle regulators, Ccnd1, Cdk4 and Cdk6, E2F transcription factor 3 (E2F3) and cell division cycle 25A (Cdc25a), to decrease osteoblast proliferation. miR-34a overexpression in MSCs subcutaneously implanted into immune deficient mice inhibited heterotopic bone formation in mice whereas anti-miR-34a overexpression had the opposite effect [48]. These studies suggest that miR-34 family miRNAs are negative regulators of bone development.

Osteogenesis is reduced upon aging, while fat accumulation in the bone marrow increases [49, 50]. A recent in vivo study showed that aging induces the expression of miR-188 in bone marrow MSCs (BMSCs) of mice and humans [51]. miR-188 KO mice showed less bone marrow adiposity and a reduction in age-related bone loss. The opposite phenotype was observed when miR-188 was overexpressed in osteoprogenitors or in BMSCs in mice. Hdac9 and RPTOR-independent companion of MTO1 complex 2 (Rictor) were identified as direct targets of miR-188. Importantly, injection of antagonomiR-188 into bone marrow stimulated bone formation and decreased marrow fat in aged mice [51]. These findings suggest that miR-188 is an important regulator of the age-related switch between osteoblast and adipocyte differentiation.

**OTHER miRNAs (STUDIED IN VITRO)**

Several other miRNAs were shown to regulate the behavior of chondrocytes, osteoblasts and their progenitors in vitro. In several studies, miRNA profiling and comparison of miRNA expression patterns were performed between cells of different stages of differentiation. These screening in vitro experiments identified microRNAs potentially important for skeletal development.

**miRNAs Regulating Chondrocytes and Their Progenitors In Vitro**

miR-675, highly expressed in human and murine articular cartilage, upregulated Col2a1 expression in human articu-
lar chondrocytes [52]. **miR-1247**, strongly expressed in mouse cartilage was found to target Sox9 [53]. Overexpression of **miR-145**, another miRNA shown to target Sox9, decreased the expression of chondrocyte marker genes during chondrocytic differentiation of a murine embryonic mesenchymal cell line (C3H10T1/2 cells). The downregulated chondrocyte markers included Col2a1, Acan, Col9a1, Col11a1 and cartilage oligomeric matrix protein (Comp). **miR-145** was downregulated during chondrocyte differentiation [54]. Similarly, **miR-145** was found to regulate human articular chondrocyte function [55]. In rat articular cartilage, **miR-337** was highly expressed at birth, but at postnatal days 21 and 42 it was significantly decreased. **miR-337** overexpression in a human chondrocyte cell line inhibited chondrocyte differentiation. Tgfbr2, encoding a receptor of transforming growth factor beta, was identified as a **miR-337** target [56]. In chicken limb mesenchymal cells **miR-375** decreased upon chondrocyte differentiation, and inhibition of **miR-375** promoted chondrogenesis. Cadherin-7, a calcium dependent cell to cell adhesion glycoprotein, was found to be a direct **miR-375** target [57]. MSC chondrocyte differentiation is inhibited by **miR-449**. In human bone marrow MSCs and in human chondrosarcoma cell lines **miR-449** reduced the expression of Col2a1 and Sox9 and proteoglycan synthesis. These actions were mediated by suppression of Lef1, encoding a transcription factor that mediates Wnt signaling [58]. During human MSC differentiation into chondrocytes, **miR-574-3p** was significantly upregulated. It was shown that Sox9 increases the transcription of **miR-574-3p** by binding to its promoter, while retinoid X receptor alpha (Rxra) was found to be a direct target of this miRNA [59]. **miR-335-5p** was also upregulated during chondrocyte differentiation of MSCs, promoting chondrogenesis. The negative regulators of Sox9, Rock1 and Daam1 were targeted by **miR-335** [60]. A recent study identified **miR-29a**, which had previously been reported to regulate osteoblast function, as an inhibitor of chondrogenesis in human MSCs. Direct targeting of Foxo3 (forkhead box O3) mediated this effect [61].

**microRNAs Regulating Osteoblasts and Their Progenitors In Vitro**

In human embryonic femurs (7-9 weeks post conception), miRNAs are differentially expressed in epiphyseal and diaphyseal cell populations [62]. Upon further analysis of miRNA expression, **miR-146b**, **miR-301** and **miR-138** showed greater expression levels in epiphyseal cells than in diaphyseal cells, while **miR-143**, **miR-145**, **miR-146a** and **miR-34a** showed greater expression in diaphyseal cells. Among these miRNAs, function of **miR-146a** was further investigated in *in vitro*. **miR-146a** was found to target Smad3, which increases Runx2 expression to promote osteogenesis [62]. **miR-146a** was previously reported to be abundantly expressed in umbilical cord MSCs [63]. **miR-146a** knockdown in these cells, inhibited their proliferation and promoted their migration [63]. Cxcl12 (chemokine ligand 12) and Sikel1, an I-kappa-B kinase epsilon (IKKe) suppressor were determined as targets of **miR-146** in the umbilical cord MSCs [63]. Thus, there may be a broader role for **miR-146** in development. Another miRNA found to increase Runx2 levels is **miR-15b**. In human bone marrow MSCs, it targeted Smurf1 encoding a protein that facilitates Runx2 degradation [64]. In contrast, **miR-338-3p**, which was downregulated during osteoblast differentiation of BMSCs, inhibited osteoblast differentiation by targeting Runx2 and Fgfr2 [65].

Several miRNAs regulate Wnt signaling. Among them are the **miR-29 family** miRNAs, which are highly conserved among vertebrates [66]. **miR-29a** suppressed the expression of the Wnt signaling inhibitors Dickkopf-related protein 1 (Dkk1), Kremen2 and secreted frizzled related protein 2 (Sfrp2), inducing osteoblast differentiation in primary human osteoblast cultures. Wnt signaling induced **miR-29a** expression, thus likely creating a positive feedback loop [67]. Rats treated with a **miR-29a** inhibitor showed a significant reduction in bone mass [68]. In a recent study, **miR-29a** was also studied in a fish bone-derived cell line (ABsa15 cells), where it was shown to increase beta-catenin levels and mineralization. The transcript of the Sparc gene, encoding osteonectin, a non-collagen ECM protein, was determined as a target of **miR-29a** [66]. **miR-29b** was upregulated during osteoblast differentiation of primary rat calvaria osteoblasts and was found to target Hdac4, Tgfbr3 (TGF-beta3), Acrv2a (activin receptor IIa), Cnnb1lp1 (catenin beta interacting protein 1) and Dusp2 (dual specificity phosphatase 2), factors that inhibit osteoblast differentiation [69]. **miR-29b** expression was found to be reduced in patients with osteogenesis imperfecta [70]. **miR-335-5p**, discussed earlier for its role in chondrogenesis, was found in osteoblasts of mouse embryos. It suppressed Dkk1 and enhanced osteogenesis. [71]. In a different study, miRNA expression was analyzed after Wnt5a stimulation *in vitro*, to investigate the effects of Wnt signaling on miRNA expression in human MSCs. **miR-141-3p** was downregulated upon Wnt signaling activation, while **miR-141-3p** overexpression inhibited Wnt5a activity and cell proliferation. It targeted Cdc25a, thus regulating cell proliferation [72]. Also, in the same study, reduction of Cdc25a was considered to mediate an inhibitory effect on osteoblast differentiation of human MSCs. In search of regulators of the Wnt signaling pathway, Wang et al. performed microarray analysis to screen for miRNAs whose expression changed during osteoblast differentiation of human BMSCs [73]. **miR-346** was identified as a promoter of osteoblast differentiation and upon its overexpression, there was a significant increase in osteoblast markers. It was shown to directly inhibit Gsk3b [73].

BMP signaling is another important pathway regulated by miRNAs in bone. **miR-497-195** cluster miRNAs are members of the miR-15 family. It is broadly expressed in tissues like lung, skin, muscle, heart and bone. Its levels were examined in mouse tibia and calvaria bones at different ages (Postnatal days P0-P42). **miR-497-195** cluster miRNAs were strongly upregulated with age during postnatal bone development [74]. They were also upregulated upon final differentiation of primary calvarial osteoblasts *in vitro*. **miR-195** acted as an intracellular antagonist of BMP signaling and inhibited osteoblast differentiation *in vitro*. Microarray and bioinformatics analysis revealed that several miRNAs encoding molecules related to BMP signaling are targets of **miR-195**; these genes include Acrv1 (activin A receptor type 1), Bmpr1a (bone morphogenetic protein receptor type 1A), Tgfbr3 (transforming growth factor, beta receptor III), Smad5 [74]. Another miRNA that regulates BMP signaling is **miR-542-3p**, **miR-542-3p** overexpression in mouse cal-

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**miRNAs in Bone Development**

*Current Genomics, 2015, Vol. 16, No. 6* 431
varial osteoblasts in vitro inhibited differentiation [75]. Bmp7 was identified as a direct target of miR-542-3p. Moreover, transfection with miR-542-3p mimic led to reductions in activity of the PI3K/Akt pathway and expression of survivin (an apoptosis inhibitor), causing caspase 3 activation and osteoblast apoptosis. Inhibition of osteoblast proliferation was also observed. In vivo treatment of ovariectomized mice with anti-miR-542-3p increased bone mass [75]. miR-140, a miRNA discussed earlier for its role in growth plate development, inhibited osteoblast lineage commitment in human MSCs by directly repressing Bmp2 [76].

FUTURE PERSPECTIVES

miRNAs have therefore, important roles in various stages of skeletal development. miRNAs and their inhibitors may be used as therapeutic options to treat skeletal disorders. miRNA mimics increase the levels of beneficial miRNAs, whereas miRNA inhibitors (antisense oligonucleotides) inhibit disease promoting miRNAs through complementary base pairing. Animal studies have shown promising results but there are still many challenges in applying miRNA based therapy in human diseases [77]. miRNAs have, often, multiple targets something that may cause several side effects. Efficient local tissue delivery and avoidance of toxicity of the nucleotide analogs are also important aspects for consideration [77]. Understanding the role of each miRNA and the effects of individual or simultaneous miRNA manipulation in vivo will facilitate the development of effective and safe miRNA-mediated therapy for developmental disorders of the skeleton.

CONCLUSIONS

Bone development is controlled by several molecules and signaling pathways and miRNAs play significant roles in this regulation. Although some in vivo studies have demonstrated important roles of miRNAs, the majority of studies has been performed in vitro, using cultured primary cells or cell lines. It is crucial to confirm these results in vivo in order to understand the physiological roles of miRNAs in bone development and identify their significance in humans. Further work is needed to translate these findings into clinical applications.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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miRNAs in Bone Development

Current Genomics, 2015, Vol. 16, No. 6 433

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