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Review Article

Role of microRNA in Mesenchymal Stem Cells Differentiation Into Osteoblasts

Aamina Shah [1], Aftab Ahmad [2,*]

[1]Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan
[2]School of Biological Sciences, University of the Punjab, Quaid iAzam Campus, Lahore, Pakistan

*Corresponding author: Aftab Ahmad, School of Biological Sciences, University of Punjab, Lahore, Pakistan. Postal Code: 54590. Tel: +92-3007402202, Fax: +92-4299230960, E-mail: aftabac@yahoo.com.

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Context: miRNAs can regulate the expression of genes by interfering in their mRNAs, acting as key regulators of diverse cellular activities. Osteoblast differentiation is an important process for skeletal developments and involves several mechanisms regulated by miRNAs. The data for this article was collected from recently published peer reviewed articles on role of miRNA in osteoblasts growth and differentiation.
Evidence Acquisition: In this review, our understanding of bone remodeling has been highlighted along with the summarized role of different miRNA that regulate mesenchymal stem cell towards osteoblast differentiation for therapeutic purposes.
Results: Treatments through miRNAs can prove to be beneficial due to its small size, easy synthesis, targeted and quick delivery.
Conclusions: Further work is needed to be done to make miRNA treatments available to large number of people suffering from various bone diseases.

Keywords: MicroRNA; Osteoblasts; Bone Remodeling; Osteogenesis

1. Introduction

Expression of gene networks are essential in regulating several cellular functions that are controlled by the coordinate act of transcription factors (TFs), RNA binding proteins and microRNAs (miRNAs) (1). RNA transcripts of genome acquires stem, loop and bulge structure, most of which behave as primary miRNA which is cut by Drosha to form pre-miRNA. Exportin-5 transfers pre-miRNA to cytoplasm (2, 3) where Dicer cuts it to create double stranded RNA (dsRNA) which is 21 - 25 mer nucleotide long and behave as miRNA by forming the RNA induced silencing complex along with the Argonaute protein (4). To some extent, miRNAs can then hybridize to mRNAs and inhibit mRNA translation (5) and promote mRNA degradation (6, 7). miRNAs are short non-coding RNAs, which are made up of ~21-nucleotides that regulate transcript localization, polyadenylation, and translation. MiRNAs act as negative regulators of gene expression directly by binding specific sequences within a target mRNA (8, 9).

In a complex functional network, miRNA acts in such a manner that the expression of one coding gene can be supervised by various miRNAs, with each miRNA possibly controlling hundreds of distinguished genes (10, 11). The role of miRNA is even evident in the regulation of numerous physiological functions like differentiation of stem cells, neurogenesis, hematopoiesis, immune response, development of cardiac and skeletal muscles, stress, etc. (12-18).

Mesenchymal Stem Cells (MSCs) known as multi-potent stromal cells isolated from various adult tissue sources have the ability to renew themselves and differentiate into multiple cell lineages (19-21). Stem cells have distinct miRNA expression profiles that can affect the intrinsic properties of self-renewal and pluripotency of stem cell (22-24).

2. Remodeling of Bone

During bone remodeling, the balance between the number and activities of osteoblasts and osteoclasts is of great significance (Figure 1). Osteoclasts have the function of bone resorption whereas osteoblasts synthesize new bones. When remodeling occurs, cytokines are released, which aid in recruiting the osteoclasts on the surface of bones. Osteoclast forms a ruffled boarder which contributes to their attachment to the surface of bone. In the cavity between the osteoclasts and the bone osteoclast's proton pump creates an acidic environment by releasing ions in the micro environment; thus, it dissolves the mineralized component of the bone matrix. The matrix gets exposed and degraded by cathepsin K (25). The

Implication for health policy/practice/research/medical education:
MiRNAs, based on the type and function, is claimed to playvarious roles in osteogenesis. Some of the miRNA behave as positive ones while others act as negative regulators during osteoblast differentiation. Understanding the mechanism of action of various miRNAs is beneficial in the perception of various bone diseases and thus development of their treatments. Treatments through miRNAs can prove beneficial due to its small size, easy synthesis, targeted and quick delivery. Further work is needed to be done to make miRNA treatments available to large number of people suffering from various bone diseases.

reversal phase of bone remodeling is when mononuclear cells transmit signal for adjustments in bone to recruit new osteoblasts. In the beginning, osteoblasts increase rapidly to release an extracellular matrix in which type I collagen is present. Along with osteoblast differentiation, the maturation and mineralization of matrix occurs. When the bone surface is restored, mature osteoblasts may undergo programmed cell death or divide to form bone surface lining cells or may even differentiate to osteocytes, present in calcified matrix and are responsive to mechanical stresses (26). Osteoclasts are derived from the monocyte/ macrophage lineage (25), whereas osteoblasts are derived from MSCs which can also differentiate into adipocytes, chondrocytes, or myocytes, depending on the activation or inhibition of specific signaling pathways (27). These signaling molecules include bone morphogenetic proteins (BMPs), transforming growth factor (TGF)-β, WNT, Hedgehog, parathyroid hormone, insulin-like growth factor-1, fibroblast growth factors and Notch.

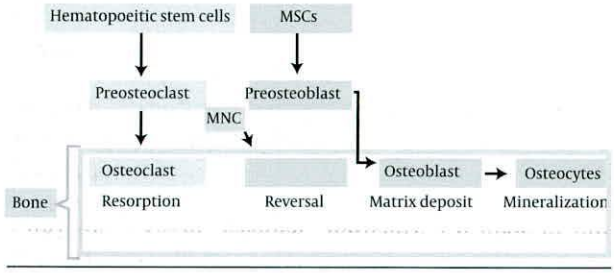

Figure 1. Bone remodeling. Haematopoietic Stem Cell-Derived Osteoclasts and Mesenchymal Stem Cell (MSC)-Derived Osteoblasts Participate in Couple and Sequential Process of Bone Remodeling.

MSCs can be isolated from bone marrow, cord blood, peripheral blood, fallopian tube, fetal liver and lung. MSCs derived from bone marrow have the potential to proliferate and get differentiated into osteoblasts, chondrocytes, adipocytes, cartilage, tendons, muscle and skin. Differentiation of MSCs into osteoblasts is very significant for bone remodeling. The differential expression of miRNAs has a major influence on the maintenance of differentiation of osteoblasts (28-30).

3. Effect of miRNA on Various Aspects of Bone Biology

3.1. Role of miRNA in Angiogenesis

Angiogenesis is a mechanism whereby regenerates and repair bones. At the time of injury, newly-formed blood vessels brings oxygen, nutrients, inflammatory cells, cartilage and bone to regenerating callus. Inflammatory cells and stromal cells produce VEGF to maintain angiogenesis. Several miRNA plays a role in angiogenesis like MiR-126, which promotes pro-angiogenesis of VEGFs and FGF by suppressing an intracellular inhibitor of angiogenic signaling known as Sprouty-related protein 1 (Spred-1) (31). In vivo developmental angiogenesis can be maintained by endothelial cell-restricted miR-126 (32). miR-210 is associated with angiogenesis in patients suffering from osteonecrosis (ON) of the femoral head (33).

3.2. Rloe of miRNA in Osteosarcoma

Osteosarcoma (OS) is a malignant bone tumor that develops during adolescence. miRNA plays a role in the down-regulation of Fas expression, in OS expression of miR-20a and miR-19 is relatively more in metastatic low-Fas-expressing LM7 cells than in the parental non metastatic high-Fas-expressing SAOS-2 cells (34). miR-125b is regulated by signal transducer and activator of transcription 3 (STAT3), which binds in vitro to the promoter region of miR-125b thus acts as a transactivator (35). BMP2 targets MiR-34c which induces changes on Notch1, Notch2 and Jag1. miR-34 and notch signaling could be used in therapies in various bone cancers (36). Down-Regulation of miR-183 promotes migration and invasion of OS by Targeting ezrin (37).

3.3. Role of miRNAs in Arthritis

Arthritis is a musculoskeletal state in which various microRNAs plays an important role. MiR-146a inhibits osteoclastogenesis and when administrated, it prevents from the destruction of joint in mice suffering from collagen-induced arthritis (CIA) by down regulating the expression of c-Jun (transcription factor), Receptor activator of nuclear factor kappa-B ligand (NF-ATc1), PU.1 (transcription factor), and tartrate-resistant acid phosphatise (TRAP) (38). MiR-223 is up- regulated in rheumatoid arthritis (RA) and lentivirus-mediated silencing of miR-223 reduces the severity of arthritis in mice (39). miR-140 is reduced in osteoarthritic chondrocytes, its knockdown promotes arthritis in mice. microRNAs should be critical factors for cartilage development and homeostasis (40). miR-221/222 and miR-323-3p are linked to rheumatoid arthritis when predicted using the human TNF in transgenic mouse model (41). Dysregulation of 17 miRNAs contribute to RA pathogenesis (42).

3.4. Role of miRNA in Osteoporosis

In Osteoporosis, bone strenght is severely affected due to which patients have higher risk of fractures. Neuronal and microRNA-dependent pathways are used for the treatment of osteoporosis (43). Various positive and negative regulator miRNA of bone remodeling can be used for therapeutic purposes of osteoporosis like miR-29 family (44), miR-138 (45), etc. miR-133a in circulating monocytes is a potential biomarker for postmenopausal osteoporosis (46). MiR-34c regulates Notch1, Notch2, and Jag1 in osteoblasts to control the differentiation of osteoclasts, presumably through cell/cell contact (35). miR-214 is elevated in patients with fractures due to low bone for-

mation rate. The inhibitory role of miR-214 in bone formation has been revealed in transgenic mice. Both the activity of osteoblast as well as mineralization of matrix promoted by antagomir-214 and deceased by agomir-214, and miR-214 inhibits osteoblast differentiation by affecting ATF4. Therefore, if miR-214 is inhibited in osteoblasts, this strategy can be used for treatment against osteoporosis (47).

Table 1. Effect of miRNAs on Various Bone Mechanisms

miRNA Involved	Mechanism	Reference
miR-126	Increase of angiogenesis	(32)
miR-210		(33)
miR-20a and miR-19a	Decrease of osteosarcoma	(34)
miR-125b		(35)
miR-34		(36)
miR-183		(37)
miR-146a	Decrease of arthritis	(38)
miR-140		(40)
miR-221/222,323-3p		(41)
miR-34c	Decrease of osteoporosis	(36)
Decreased miR-214		(47)

4. miRNAs as Regulators of Osteoblast Differentiation

4.1. miRNAs as Negative Regulators

Several miRNAs regulates bone remodeling by acting as negative regulators of osteoblast differentiation. miR-206 connexin 43 (Cx43) is one of the gap junction protein found in osteoblasts and its negatively regulated if miR-206 is overexpressed (30). miR-378 inhibits osteoblast differentiation by reducing the expression of nephronectin (NN, an extracellular matrix protein) (48). Osteoblast differentiation is also inhibited by miR-138 that targets FAK (Focal Adhesion Kinase) translation, consequently reducing phosphorylation of FAK. Its target ERK1/2 proteins results in decreased phosphorylation of Runx2 and Osterix expression 45.

4.2. Effect of miRNA on Runx2 in Negative Osteogenesis

Runx2 is a bone specific transcription factor that regulates several genes and is required for the expression of several osteoblast differentiation genes such as type I collagen, osteocalcin (49). Runx2 gene expression is inhibited by miR-204 and miR-211, which behaves as negative regulators for the differentiation of osteoblasts and mineralization in mesenchymal progenitor cells and bone marrow stromal cell (BMSCs) (50). Various other miRNAs like miR-23a, miR-30c, miR-34c, miR-133a, miR-135a, miR-137, miR-204, miR-205, miR-217, and miR-338 also target and decrease the expression of Runx2 leading to the inhibition of osteoblast differentiation (51, 52). miR-355 also targets Runx2 mRNA and when miR-335 is overexpressed in hMSCs, it inhibits proliferation, migration as well as osteogenic and adipogenic potential (53). MSCs differentiation into osteoblasts can be inhibited by miRNAs such as hsa-miR-31, hsa-miR-106a, hsa-miR-148a, and hsa-miR-424 and their target genes are Runx2, Cbfb and BMPs. miR-133 and miR- 135 inhibit the differentiation of osteoprogenitors by acting on Runx2 and Smad5 pathways (54). miR-26a also inhibits osteoblast differentiation by targeting Smad1 in human adipose tissue derived stem cells (55). Smad proteins are intracellular signaling components for TGF-β/ BMP signaling and BMP-2 via Smads activates osteoblast essential genes (56). TRPS1 is a chondrogenic GATA transcription factor controlled by miRNAs (51, 52, 54). Due to mutation in Runx2 gene, dominantly inherited skeletal malformation cleidocranial dysplasia (CCD) occur. Mutations in the human Trps1 gene causesTricho-Rhino-Phalangeal syndrome types I and III (57-59). Ten Runx2-targeting miRNAs blocks MC3T3 osteoblast differentiation (52). Seven of these also targets TRPS1 and miR-30c is such a TRPS1/RUNX2-targeting miRNA. TRPS1 inhibits the transcriptional activity of RUNX2 along with Runx2 promoter (58). The genetic interactions and biological correlations between RUNX2 and TRPS1 are remarkable, since they have common miRNAs that regulate their expression, which block their osteochondrogenic functions, the activities of RUNX2 and TRPS1 leading to a common function linked to early stages of differentiation in skeletal cells (60).

Treatment of osteoblastic or chondrocytic cells with a representative TRPS1/RUNX2-targeting miRNA (miR-30c) results in opposite and coordinate effects on the expression of a number of genes encoding components of signaling pathways (e.g. responding to TGF-β/BMP2, IGF1 or FGF2) that control bone or cartilage development (61-63). miR-30 also up-regulates TGF-β signaling components while osteoblast differentiation and the resulting sensitization of TGF-β/SMAD3 signaling synergize with miR-30c to repress RUNX2 and the expression of osteoblast specific ECM proteins (60).

PTK2 encodes FAK protein, which is the activator of ERK1 and ERK2 during osteoblast differentiation of hMSCs, miR-138 binds to 3´UTR of PTK2 and acts as negative regulator of hMSCs by maintaining its undifferentiated state (64, 65). The overexpression of miR-138 diminishes FAK at the protein level, whereas the inhibition of miR-138 by antimiR-138 causes the depression of FAK, which is regulated by miR-138 during osteoblast differentiation (66).

4.3. miRNA as Positive Regulators

Certain miRNAs act as positive regulators of osteogenic

differentiation like miR-29b down-regulates inhibitors of osteoblast differentiation such as HDAC4, TGF3, ACVR2A, CTNNBIP1, and DUSP2; thus, it promotes osteogenesis (67, 68). Increased levels of let-7, miR-24, miR-125b, and miR-138 repress the expression of non osteogenic target mRNAs involved in PDGF pathway, so these miRNAs up-regulates osteogenic differentiation (69). miR-218 acts as positive regulators during the differentiation of osteoblasts to the final stage of forming mineralized tissue by inhibiting ERBI (TOBI) and sclerostin (SOST) (70). When the same miRNA polycistron causes transcription of miR-3960 and miR-2861, they act on Runx2/miR-3960/miR-2861 regulation feedback mechanism and help in osteoblast differentiation (71). miR-26a/b and miR-29b both behave as positive regulators of osteogenic differentiation in unique stem cell type (USSC) by acting on CDK6 and HDAC4 which are osteoinhibitory proteins (72).

4.4. Effect of miRNA on Runx2 in Positive Osteogenesis

During positive osteogenesis, RUNX2 is influenced by miRNAs like the expression of miR-20a increasing during the osteogenic differentiation and it stimulates the osteogenesis of hMSCs. BMPs and Runx2 are important factors in the osteogenesis of MSCs (8, 73). miR-20a suppresses PPARγ, which is a very important factor for the differentiation of osteoblasts and also is essential for maintaining homeostasis of bones (74, 75). PPARγ is the antagonist of BMP and RUNX2 especially when the activated inhibits RUNX2 transcription (76) and down regulates the expression of BMP (77). Due to lowered action of BMP and RUNX2 (77), the phenotypic expression of osteoblast is repressed by PPARγ. Antagonists of the BMP signaling pathway are generally categorized at three levels: extracellular (Noggin, Gremlin, Chordin), cell surface (Bambi and Crim1) and intracellular inhibitory (Smads 6 and 7) (78) .Binding sites miR20a are also present in 3'-UTR of Bambi and Crim1. Bambi is negatively activated on by miR-20a and more BMP molecules bind to their receptors, thus, the RUNX2 expression is increased, whereas the role of Crim1 on BMPs after binding them is to reduce their secretion as mature active proteins and processing of pre-protein to mature BMP; hence, it causes the binding of pre-proteins to cell surface (73, 79) and suppression of Crim1 occuring after the transfection of miR-20a. therefore, there is an increase of free, mature and active BMP that activate BMP signaling, thus enhancing the osteogenesis of MSCs (73).

4.5. OstemiR and MSCs Differentiation Into Bone

OstemiR includes miR-30 family, let-7 family, miR-21, miR-16, miR-155, miR-322 and Snord85, whose expression alters during osteogenic differentiation. They play a role in stemness, epinegetics, and cell cycle-related mRNAs (80).

Different miRNA have different roles in osteogenesis. Some of the miRNA behave as positive regulators; while others act as negative regulators during osteoblast differentiation. Understanding the role of various miRNAs is beneficial in understanding the various bone diseases and thus the development of their treatments. Treatments through miRNAs can be beneficial, due to its small size, easy synthesis, targeted and quick delivery. Further work needed to be done to make miRNA treatment available to large number of people suffering from various bone diseases.

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Authors' Contribution

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