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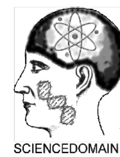

MicroRNAs and Their Role in Bone Remodeling and Pathogenesis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AS and SA designed whole of the idea and wrote the first draft of the manuscript. Authors AAM and RNS managed the literature searches. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

MicroRNAs, a class of post-transcriptional gene expression regulators that bind to complementary sequences in the 3' UTR or 5' UTR of mRNAs have recently been detected in human body fluids including peripheral blood plasma as extracellular nuclease resistant entities. It is now clear that the biogenesis and functions of microRNAs are related to the molecular mechanisms of various clinical diseases and they can potentially regulate every aspect of cellular activity. This review will highlight our current understanding of microRNA biogenesis and their mechanisms of action. It will also summarize recent works on the role of microRNAs in bone remodeling including angiogenesis, osteoblast and osteoclast differentiation and in various bone related pathologies. An in-depth understanding of the roles of these regulatory mRNAs in the skeleton will be critical for the development of new therapeutics aimed on bone remodeling including fracture repair and bone-related diseases.

Keywords: MicroRNA; biogenesis; expression; bone remodeling; pathogenesis.

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ABBRIVATIONS

MicroRNAs – miR; ECM - Extracellular matrix; RANKL - Receptor activator of nuclear factor kappa-B ligand; M-CSF - macrophage colony- stimulating factor; TRAP - Tartrate-resistant acid phosphatise; CTR - Calcitonin receptor; pri-microRNA - Primary microRNA; RISC-RNA-induced silencing complex; Ago2 - Argonaute protein; GCR8 - Di George Syndrome Critical Region 8; NFI-A - Nuclear factor 1 A-type; M-CSFR - Macrophage colony-stimulating factor receptor; VEGF - Vascular endothelial growth factor; FGF - Fibroblast growth factors; Spred-1- Sprouty-related protein 1; MG-63 osteoblast-like cell line; BO - Bio-Oss; PG - PerioGlass; HMSC - Human mesenchymal stem cell; qRT-PCR - quantitative Real time Polymerase Chain Reaction; RA- rheumatoid arthritis PBMCs- Peripheral blood mononuclear cells; CIA- collagen-induced arthritis; c-Jun -Transcription factor; NF-ATc1-Receptor activator of nuclear factor kappa-B ligand; PU.1-transcription factor; HDAC5-Histone deacetylase 5; Runx2 - Runt-related transcription factor 2; BMP2-Bone morphogenic protein 2; Hoxa2 - Homeobox A2; PDCD4- Programmed cell death 4; c-Fos - A critical transcription Factor; MC3T3-E1 -Bone cells; AgNPs- Silver nanoparticles; PDLSCs - Periodontal ligament stem cells; ALP - Alkaline phosphatase; COL-1-Type I collagen; OPG- Osteoprotegerin; OCN-Osteocalcin; 3'-UTRs - 3'-untranslated regions; FGF2-Fibroblast growth factor 2; BMD- Bone mineral density; Dkk1- Dikkopf-1; sFRP2-Secreted frizzled related protein 2 ; Cx43- Connexin 43; N.Oc/BS- Number of osteoclasts; Oc.S/BS - Osteoclast surface; MAR - Mineral apposition rate BFR -Bone formation rate; Efnb2 - Ephrin-B2 protein; SCs - Stem cells; USSCs-Unrestricted somatic stem cells; AcvR1b -Activin A receptor type 1B ; TGF-beta - transforming growth factor-beta; ST2 cells-Stromal cell line ; SB431542 - Inhibitor of the activin receptor; ACVR2A - Activin receptor type-2A gene; CTNNBIP1 - Catenin, beta interacting protein 1; DUSP2 - Dual specificity protein phosphatase 2 ;PP - Porous polyethylene; MG-63- Osteoblast-like cells line; AC-Anatase coating; hADSCs - Human adipose tissue-derived stem cells; SMAD1 - Transcription factor; BMPs - Bone morphogenetic proteins; OA - Osteoarthritis; TGF- - Transforming growth factor ; IL-11 - Interleukin 11; PTGS2 - Prostaglandin-endoperoxide synthase 2; Ets1 V-ets- Erythroblastosis Virus E26 Oncogene Homolog 1; SOX9-Transcription factor; FABP4- Fatty acid binding protein 4; ROS- Reactive oxygen species, STAT3- Signal transducer and activator of transcription 3; WJ-MSCs-Wharton's jelly matrix of human umbilical cord; Osx- Osterix; AD - Adipocyte differentiation; ALPL- Alkaline phosphatase liver/bone/kidney; SATB2 - Special AT- rich sequence-binding protein 2; STAT3- Signal transducer and activator of transcription 3; Ago - Argonaute; PACT - Protein activator of PKR; P-body - Processing body; MARs - Matrix-attachment regions.

1. INTRODUCTION

MicroRNAs are a recently discovered family of endogenous, non-coding single strand RNAs molecules approximately 22 nucleotide in length. MicroRNAs modulate gene expression post transcriptionally by binding to complementary sequences in the coding or 3 untranslated region of target messenger RNAs (mRNAs). It is now clear that the biogenesis and function of microRNAs are related to the molecular mechanisms of various clinical diseases and that they can potentially regulate every aspect of cellular activity including differentiation and development, metabolism, proliferation, apoptotic cell death, viral infection and tumorgenesis (Yong et al., 2011). While they were studying gene lin-14 in C.elegans, Lee et al (1993) first identified microRNA, but the term microRNA was coined in 2001 by Ruvkun. Lee et al. found that LIN-14 protein abundance was regulated by a short RNA product encoded by the lin-4 gene. Since then hundreds of microRNAs have been

identified from plants, animals and viruses. Consecutively, microRNAs have proven to play essential roles in diverse biological processes including early foetal development (Grififiths et al., 2006), cell proliferation (Reinhart et al., 2000), cell death (Lu et al., 2005), fat metabolism (Brennecke et al., 2003), cell differentiation (Xu et al., 2003; Dostie et al.,2003; Chen et al., 2004), osteogenesis (Sugatani et al., 2009; Sun et al., 2011; Kapinas et al., 2011; Lei et al., 2011; Eskildsen et al., 2011; Li et al., 2009) and brain development (Sugatani et al., 2009). The sequence codings for microRNAs are spread around the genome, including exons, introns, 3'-UTRs and genomic repeat-areas and are situated either in the sense or antisense orientation with respect to the overlapping protein-coding gene (Houbaviy et al., 2003). As we discussed above that most of the microRNA binding sites lie within the 3' UTR, there are some reports of microRNAs binding in the 5' UTR and coding region of mRNAs (Lytle et al., 2007; Duursma et al., 2008). It seems to be a biological basis for the preferential interaction of microRNAs with the 3' UTR. Some data suggest that microRNA binding sites within the coding region of a transcript are less effective at mediating translational repression. This is likely due to the ability of ribosomal complexes to override and inhibit the interaction of the microRNA-RISC complex with the potential binding sites (Gu et al., 2009). Similarly, relatively few functional microRNA binding sites are located in the 5' UTR of a transcript. The scanning activity of the ribosome may impair the interaction of the microRNA-RISC complex with the 5' UTR, suggesting that there would be inefficient inhibition of gene expression. Although the general location of a microRNA binding site within the transcript, helps to define the degree of repression mediated by microRNAs, other factors such as the sequence context of the microRNA binding site, the number of target sites within the microRNA, the local RNA structure, and distance between target sites; contribute to efficacy (Lewis et al., 2005; Brenneke et al., 2005; Long et al., 2007; Saetrom et al., 2007).

The skeleton is continuously remodeled throughout the lifetime of an individual via dynamic cycle of formation, mineralisation and resorption. This remodeling process consists of a homeostasis of bone cells, mediated by the delicate balance of osteoblast and osteoclast numbers and activities (Raisz et al., 1999; Ross et al., 2008). Osteoclast begins this cycle by resorbing bone minerals and matrix. Mononuclear cells prepare the surface for osteoblasts, which differentiate into newly synthesized matrix. Remodeling cycle completes on matrix mineralization and differentiation of some osteoblast into osteocytes. Misregulation of osteoclastic or osteoblastic differentiation could result in dysregulation of bone balance with pathological consequences.

Several microRNAs regulate the proliferation and differentiation of osteoblasts, osteoclasts and chondrocytes, eventually influencing bone formation and its metabolism. MicroRNAs are expected to provide potential gene therapy targets for the clinical treatment of metabolic bone diseases and bone injuries (Dong et al., 2012). Laine et al. in a review summarizes the current insights into microRNA-mediated regulation of bone marrow stem/progenitor cell maintenance and differentiation (Laine et al., 2012). MicroRNAs repress cellular protein levels to provide a sophisticated parameter of gene regulation that coordinates a broad spectrum of biological processes. Bone organogenesis is a complex process involving the differentiation and crosstalk of multiple cell types for formation and remodeling of the skeleton. Inhibition of mRNA translation by microRNAs has emerged as an important regulator of developmental osteogenic signaling pathways, osteoblast growth and differentiation, osteoclast-mediated bone resorption activity and bone homeostasis in the adult skeleton. MicroRNAs control multiple layers of gene regulation for bone development and postnatal functions, from the initial response of stem/progenitor cells to the structural and metabolic activity of the mature tissue (Lian et al., 2012). Although various microRNAs

regulate cell proliferation and differentiation of osteoblast (Kapinas et al., 2011), few microRNAs have been reported to play a key role in the regulation of osteoclast differentiation (Xia et al., 2011). The goal of this review is to highlight our current understanding of microRNA biogenesis and their mechanisms of action and to summarize recent studies on the role of microRNAs in bone pathogenesis and remodeling.

2. BONE FUNCTION AND STRUCTURE

Instead of being an inert and static material, bones are highly organized living tissue and are the major constituent of the musculoskeletal system. Main functions of bones are to protect internal organs and to support the body structures. Beyond these functions, bones additionally act as an attachment site for muscles allowing locomotion and as an appropriate cavity for hematopoiesis in bone marrow. As a reservoir for inorganic ions, bone is responsible for the maintenance of calcium homeostasis and is able to rapidly mobilize mineral stores on metabolic demand. Bone consists of cells and extracellular matrix (ECM), the latter being further subdivided into an inorganic and organic part. The organic matrix is mainly consist of type I collagen (approximately 95%), as well as other types of collagens, non collagenous proteins and proteoglycans, whereas the inorganic matrix predominantly contains calcium and phosphorus, appearing as hydroxyapatite crystals ([3Ca 3 (PO 4) 2] (OH) 2) deposited into the collagenous matrix. This interdigitate organization confers rigidity and strength to the skeleton while maintaining a certain degree of elasticity. The major cells in bone are the osteoclasts (bone resorbing tissue), osteoblasts (bone depositing tissue) and osteocytes (bone lining cells).

3. OSTEOBLASTS AND OSTEOCLASTS

The development and homeostasis of the vertebrate skeletal system depends on a dynamic balancing of the activities of bone forming osteoblast and bone resorbing osteoclasts (Fig. 1). Osteoblasts produce a variety of proteins for bone matrix synthesis and having a prominent golgi apparatus and rough endoplasmic reticulum. Whereas osteocytes have poor inorganelles, indicating their other primary functions than matrix synthesis and mineralization. In fact, mature osteocytes alter their morphology by forming dendritic processes which enable them to communicate with other embedded osteocytes (Noble et al., 2003; Rawlinson et al., 1996; Marroti et al., 1990; Mullender et al., 2005). Apoptotic osteocytes increase the secretion of osteoclastogenic cytokines and thereby enhance bone resorption (Gu et al., 2005; Kogianni et al., 2006). Bone lining cells arise from osteoblasts and are believed to be resting, inactive osteoblasts, covering the bone surface. It has been observed that an important role in the initiation of bone remodeling has been assigned to them (Miller et al., 1989). Osteoclasts are the only cells capable of breaking down mineralized bone, dentine and calcified cartilage (Udagawa et al., 1990; Kurihara et al., 1990). The presence of Receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony- stimulating factor (M-CSF) are essential for the formation and fusion of multinucleated cells, expressing osteoclast specific markers such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, calcitonin receptor (CTR) and integrin receptors (Kodama et al., 1991; Jacquin et al., 2006; Luchin et al., 2000; Hattersley et al., 1989; Hansen et al., 2001; Teti et al., 1989).

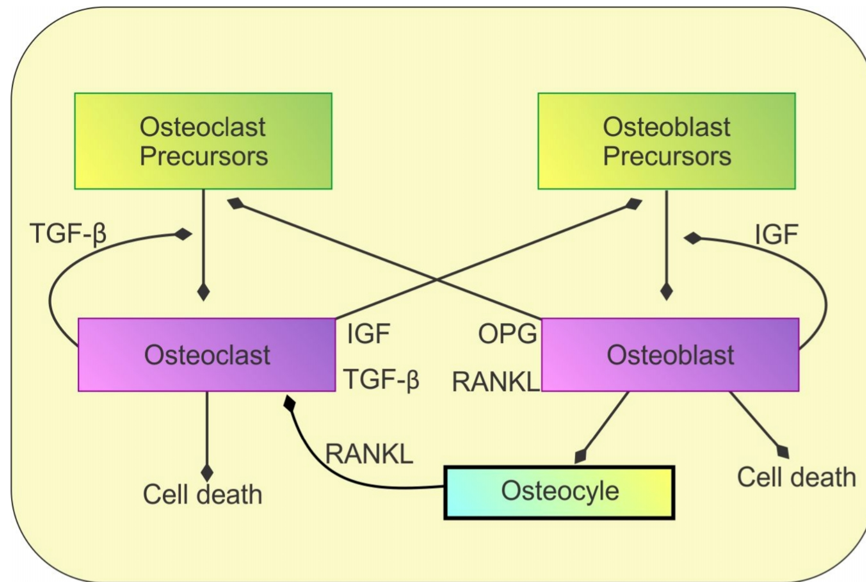

Fig. 1. A schematic demonstration of bone signaling, web of feedback loops involved in damage stimulated bone remodeling

4. BIOGENESIS OF MICRO-RNAs

The microRNA biogenesis is a complex multi-step process starting in the nucleus and ending in the cytoplasm, with many post-transcriptional modifications. The initiation of transcription mostly carried out by RNA polymerase II, generating a primary microRNA (pri-microRNA), characterized by a hairpin RNA structure recognized by the nuclear RNAse III enzyme Drosha, and its cofactor DGCR8 (Liu et al., 2009; Seitz et al., 2006). These proteins work within a complex of several proteins known as the Microprocessor, which cleaves the pri-microRNA to generate a shorter hairpin of about 70 nucleotide length—the pre-microRNA.

The pre-microRNA is then transported from the nucleus into the cytoplasm by Exportin5 and Ran-GTP (Lund et al., 2004). Exempted from this processing pathway 'mirtrons', a short introns containing microRNA precursors, spliced and debranched and further bypass the processing by Drosha and access the canonical microRNA processing pathway following nuclear export (Berezikov et al., 2007). Dicer-TAR RNA binding protein (TRBP) complex further processed the Pre-microRNAs (Chendrimada et al., 2005). Dicer is another RNAase III-type endonuclease and TRBP is thought to recruit and bind the pre-microRNA, and to stabilize the Dicer-RNA interaction (Fig. 2). There are various requirements for duplex recognition and cleavage by Dicer (Kawamata et al., 2009). Dicer cleavage yields an approximately 21-nucleotide microRNA duplex, which is incorporated into the RNA-induced silencing complex (RISC). The RISC includes Dicer, TRBP, PACT (protein activator of FPKR), and one of four Ago proteins (Landthaler et al., 2008). The microRNA duplex is unwound by helicases into two single strands, the mature guide strand (microRNA; in red strand) and the complementary passenger strand (Kawamata et al., 2009). Guide strand selection is dependent on the first 5' nucleotides and which Ago protein is present in the RISC (Takeda et al., 2008). Translational repression of m7G mRNAs occurs via eIF4E suggesting that Agos can compete with eIF4E to inhibit translation (Kiriakidou et al., 2007).

As a component of the RISC, Ago proteins 1, 3, and 4 are thought to mediate post-initiation inhibition by promoting poly (A) tail-mediated degradation (Battacharyya et al., 2006; Olsen et al., 1999; Petersen et al., 2006). Further, microRNAs function in P-bodies by sequestering target transcripts for storage, decapping, deadenylation, and degradation (Liu et al., 2005). Interestingly, P-bodies may also act as a temporary storage space for translationally repressed mRNAs. Since most P-body components are also found dispersed in the cytosol, it is likely that repression by these proteins is initiated in the cytosol, and the repressed mRNAs then aggregate to form the P-body (Battacharyya et al., 2006).

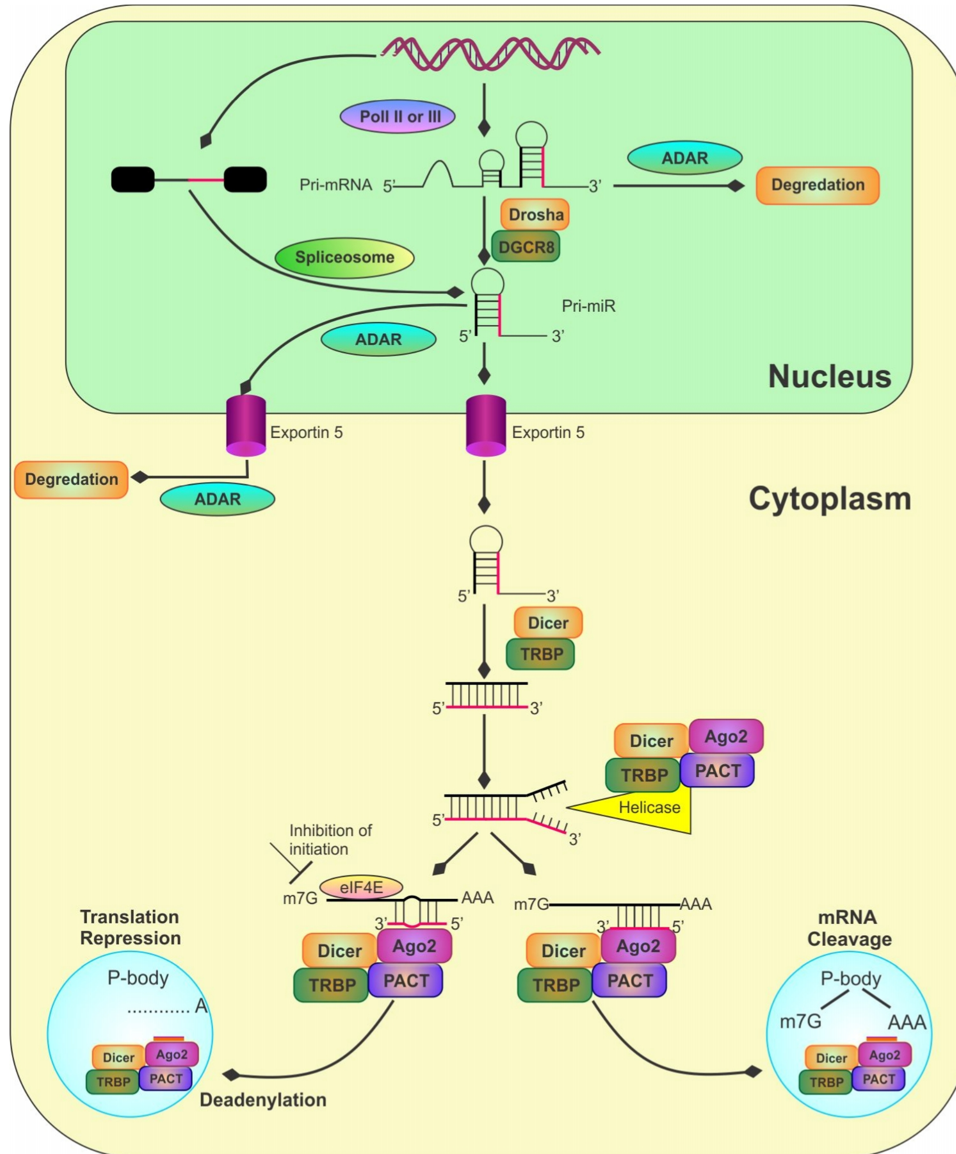

Fig. 2. The microRNA biogenesis pathway

While the mechanisms regulating microRNA biogenesis have been well studied, there have requirement to understand the regulation of microRNA stability and characterization of *cis*- and *trans*-acting factors regulating microRNA stability, particularly in vertebrates.

5. MICRO-RNAs IN SKELETOGENESIS

Skeletogenesis is a tightly regulated process during fetal, postnatal and pubertal growth, which is finely orchestrated by transcription factors and consecutive signaling pathways. It is controlled by delicate feedback loops. Most skeletogenic cells later develop into cartilage cells (chondrocytes), bone cells (osteoblasts), or joint cells (mainly articular chondrocytes and synovial cells), while some may persist as mesenchymal stem cells throughout life. The primary skeleton is entirely cartilaginous and most of it is progressively replaced by bone throughout fetal and postnatal growth. This process is called endochondral ossification. Concomitantly, joints and additional bones form. The latter develop upon a mesenchymal template, without cartilage intermediate, through a process called intramembranous ossification. Bone, cartilage, and joints differ in composition and regulation, but their associated developments are tightly coordinated. Our knowledge of the cellular and molecular events that govern skeletogenesis has greatly increased over the last two decades. It is now clear that an amazingly large number of factors are involved in skeletogenesis. Recently microRNAs have been found as one of the important factor in skeleton development in evolution, health, and disease. Selenium participates in the formation of selenoprotein as the form of selenocysteine, the active-site residue essential for protein catalytic activity. It has been proved that selenium deficiency leads to the abnormality of selenoprotein biosynthesis, which may lead to disorders of brain, skeleton development and chondrocyte differentiation. Recent evidence indicates that microRNAs are key points in the process of endochondral ossification and the expression of key selenoproteins may regulate directly or indirectly via trans-acting factors by microRNAs during skeleton development (Sun et al., 2011). Liu et al. (2009) in a review concentrated on the roles of microRNAs in skeletogenesis as well as in skeleton-related disease processes. In the osteogenic differentiation process of hBMSCs, hsa-miRNA-149 and hsa-miRNA-654-5p are closely related with the mRNA and protein regulation of ALPL and BMP-2, respectively (Wei et al., 2012). They also perform in-vivo study in mouse to reveal that miR-34b and miR-34c affected skeletogenesis during embryonic development, as well as bone mass accrual after birth. It is done firstly by inhibiting osteoblast proliferation by suppressing Cyclin D1, CDK4, and CDK6 accumulation and secondly, by inhibiting terminal differentiation of osteoblasts via inhibition of SATB2, a nuclear matrix protein that is a critical determinant of osteoblast differentiation. Genetic evidence obtained in the mouse confirmed the importance of SATB2 regulation by miR-34b/c. Thus, it would be vital firstly to identify a family of microRNAs involved in bone formation in vivo and further to identify a specific genetic pathway by which these microRNAs regulate osteoblast differentiation. Despite the identification of miRNAs, expressed with exquisite precision in skeletal tissues, we still know little about how they affect skeletal development. Finally, although miRNAs can phenocopy human skeletal disease in model organisms, none has yet been directly linked to human skeletal disease. The promise of miRNA mimics or inhibitors as systemic therapies of disease promises should propel the intensive investigation of the roles of miRNAs in skeletal disease.

6. ROLE OF MICRO-RNAs IN BONE REMODELING

The skeleton is a metabolically active organ that undergoes continuous remodeling throughout life. Bone remodeling serves to adjust bone architecture to meet changing mechanical needs and it helps to repair microdamages in bone matrix preventing the accumulation of old bone. It also plays an important role in maintaining plasma calcium homeostasis (Hadjidakis et al., 2006). As bone is a highly vascularised tissue reliant on the close spatial and temporal connection between blood vessels and bone cells to maintain skeletal integrity. Angiogenesis thus plays a pivotal role in skeletal development and bone fracture repair (Kanczler et al., 2008). Bone remodelling, or turnover, is mediated by the delicate balance of osteoblast and osteoclast numbers and activities. Osteoclasts resorb bone, whereas osteoblasts synthesize new bone. The release of cytokines at the site of remodelling recruits osteoclasts to the bone surface. The osteoclasts form a ruffled boarder that provides their tight adherence to the bone surface. The space between the osteoclast and the underlying bone becomes an isolated microenvironment into which the osteoclast's proton pump releases ions that generate an acidic environment, dissolving the mineralized component of the bone matrix. The cathepsin K subsequently degrades exposed organic matrix (Ross et al., 2008). The mononuclear cells begin the reversal phase of bone remodelling and preparing the bone surface for new osteoblasts. Early osteoblasts proliferate and secrete an extracellular matrix abundant in type I collagen. As the osteoblasts continue to differentiate, the matrix matures and is mineralized. Once the bone surface is restored, mature osteoblasts can undergo apoptosis or terminally differentiate into either bone surface lining cells or osteocytes, which are embedded in the calcified matrix and are responsive to mechanical stresses (Bonewald et al., 2008). Osteoclasts and osteoblasts differentiation from multipotent precursors is a critical component of bone turnover. Osteoclasts differentiation is dependent on multiple extracellular signalling molecules, including macrophage colony-stimulating factor (MCSF), receptor activator for nuclear factor B ligand (RANKL), tumour necrosis factor, interferon gamma, and interleukins (Ross et al., 2008). Osteoblasts are differentiating into osteoblasts, adipocytes, chondrocytes, or myocytes, depending on the activation or inhibition of specific signalling pathways (Krause et al., 2008). Some of the most important signalling molecules regulating osteoblastic differentiation include bone morphogenetic proteins (BMPs), transforming growth factor (TGF)- , WNT, Hedgehog, parathyroid hormone, insulin-like growth factor-1, fibroblast growth factors, and Notch. Misregulation of osteoclastic or osteoblastic differentiation could result in dysregulation of bone balance and pathological consequences, including osteoporosis. MicroRNAs have been shown to regulate osteoblast and osteoclast differentiation and function. The following section will summarize the known roles of microRNAs in osteoblast and osteoclast biology along with bone pathogenesis.

6.1 MicroRNAs in Angiogenesis

Angiogenesis is a key component of bone regeneration or bone repair. New blood vessels bring oxygen and nutrients to the highly metabolically active regenerating callus and serve as a route for inflammatory cells and cartilage and bone precursor cells to reach the injury site. Angiogenesis is regulated by a variety of growth factors, notably vascular endothelial growth factor (VEGF), which are produced by inflammatory cells and stromal cells to induce blood vessel in-growth. MiR-126 enhances the proangiogenic actions of Vascular endothelial growth factor (VEGF) and Fibroblast growth factors (FGF) and promotes blood vessel formation by repressing the expression of Sprouty-related protein 1 (Spred-1), an intracellular inhibitor of angiogenic signaling (Fish et al., 2008). During vertebrate

embryogenesis, vascular patterning is guided initially by conserved genetic pathways that act before circulation. Wang et al in same year also found that an endothelial cell-restricted miR-126 mediates developmental angiogenesis *in vivo* (Wang et al., 2008). The expression pattern of miR-210, recently also known to be associated with angiogenesis, in bone from patients with osteonecrosis (ON) of the femoral head. This study showed that miR-210 is intensely expressed in ON, and might play a role in ON pathogenesis (Yamasaki et al., 2012).

A variety of studies in transgenic and gene-targeted mice have demonstrated the importance of angiogenesis in fracture healing, and have provided insights into regulatory processes governing fracture angiogenesis. Indeed, in animal models enhancing angiogenesis promotes bone regeneration, suggesting that modifying fracture vascularisation could be a viable therapeutic approach for accelerated/improved clinico-radiological bone regeneration.

6.2 MicroRNAs in Osteoblast/Osteoclast Function

Osteoblast differentiation is a key step in proper skeletal development and acquisition of bone mass. Bone and cartilage are being generated de novo through concerted actions of a plethora of signals. The signals are rendered by hormones and growth factors (GFs) and mechanical forces ensuring proper modeling and remodeling of bone and cartilage, due to indigenous and programmed metabolism in stem cells (SCs), osteoblasts, chondrocytes, as well as osteoclasts and other cell types (e.g. T helper cells). Gordeladze et al. focuses on the concerted action of such signals, as well as the regulatory and/or stabilizing control circuits rendered by microRNAs (Gordeladze et al., 2009). Early study shows that microRNAs expression was significantly modified in an osteoblast-like cell line (MG-63) cultured with Bio-Oss (BO) v/s PerioGlass (PG). Three up-regulated microRNAs (miR-337, miR-200b, and miR-377) and 4 down-regulated microRNAs (miR-130a, miR-214, miR-27a, and miR-93) were identified. These results indicated that PG causes activation of bone-forming signaling, whereas BO also activates cartilage-related pathways (Annalisa et al., 2008). Porous polyethylene (PP or Medpor) is an alloplastic material worldwide used for craniofacial reconstruction. Palmieri et al. (2008) identified the osteoblast-like cells line (MG-63) cultured with Medpor, the expression of which is significantly modified by microRNA. They identified 16 up-regulated microRNA (i.e. miR-337, miR-515-3p, miR-377, miR-153, miR-367, miR-152, let-7b, miR-92, miR-155, miR-424, miR-148b, miR-368, miR-18b, miR-520d, miR-20b, and miR-128a) and 2 down-regulated microRNA (i.e. miR-143, miR-32) in this series. It has been established that Human adipose tissue-derived stem cells (hADSCs) do differentiate toward osteogenic precursors and subsequent bone-forming osteoblasts. Using osteoblast precursors, obtained from subcutaneous human adipose tissue, it was observed by Luzi et al. (2008) that microRNA-26a (Table-1) modulate late osteoblasts differentiation, by targeting the SMAD1 transcription factor. Though microRNAs have spatio-temporally modulated gene expression; however, very little is known about the regulation of their expression. Pavithra et al. (2011), in a study found that, the well-known *cis*-regulatory elements of gene expression, scaffold/matrix-attachment regions (MARs) could modulate microRNA expression. Manipulation of microRNAs activity accelerates osteogenic differentiation of hMSCs in engineered synthetic 3D scaffold tissue give similar responses to soluble osteogenic signals. They suggested that transfected hMSCs with specific microRNA can be an effective tool for enhancing the induction of osteogenesis for tissue engineering purposes (Mariner et al., 2012).

Table 1. A summary of some of the current correlative and causal relationship elucidating between microRNA and osteogenesis, bone-remodeling and bone disease

MicroRNA(s)	Implication of microRNA involvement in bone metabolism and disease	References
miR-223	Lentivirus-mediated silencing of miR-223 can reduce disease severity of arthritis.	Li et al. (2012)
miR-379	Inhibit TGF- -Induced IL-11 Production in Bone Metastatic process in Breast Cancer Cells.	Pollari et al. (2012)
miR-370	Regulates the expression BMP-2 and Ets1 in BMP-2-stimulated murine pre-osteoblast MC3T3-E1 cell differentiation.	Itoh et al. (2012)
hsa-miR-149,654-5p	In the osteogenic differentiation process of hBMSCs, negatively correlated with the mRNA and protein regulation of ALPL and BMP-2.	Wei et al. (2012)
miR-34s	Inhibit osteoblast proliferation by suppressing Cyclin D1, CDK4, and CDK6 accumulation and terminal differentiation through the inhibition of SATB2.	Wei et al. (2012)
miR-140	Expressed in chondrocytes and its expression get reduced in OA chondrocytes, knockdown of miR-140 in mice chondrocytes promotes arthritis in mice.	Asahar et al. (2012)
miR-183	Down-Regulation of miR-183 Promotes Migration and Invasion of Osteosarcoma by Targeting Ezrin.	Zhu et al. (2012)
miR-133a	miR-133a in circulating monocytes is a potential biomarker for postmenopausal osteoporosis.	Wang et al. (2012)
miR-34c	Targets multiple components of the Notch signalling pathway, including Notch1, Notch2 and Jag1 and influences osteoclast differentiation during bone development.	Bae et al. (2012)
miR-93	Important regulator in osteoblast mineralization and function through a novel miR-93/Sp7 regulatory feedback loop.	Yang et al. (2012)
miR-9	Promotes the neural differentiation of mouse bone marrow mesenchymal stem cells via targeting zinc finger protein 521.	Han et al. (2012)
miR-96, 124, 199a	Regulated the expression of genes important for hMSC differentiation, such as aggrecan, transcription factor SOX9 and fatty acid binding protein 4 (FABP4).	Laine et al. (2012)
miR-182	Negative regulator of osteoblast proliferation, differentiation and skeletogenesis through targeting FoxO1.	Kim et al. (2012)
miR-26a	Positive regulator of MEN1 mRNA, with a consequent up-regulation of SMAD1 protein.	Luzi et al. (2012)
miR-210	Known to be associated with angiogenesis and intensely expressed in ON patients.	Yamasaki et al. (2012)
miR-30 family	Negatively regulate BMP-2-induced osteoblast differentiation by targeting Smad1 and Runx2.	Wu et al. (2012)
miR-20a	Encoded by the miR-17-92 cluster increases the metastatic potential of osteosarcoma cells by regulating Fas expression.	Huang et al. (2012)

DGCR8, Dicer, and Ago2 are essential factors for microRNA homeostasis, with critical roles in osteoclast differentiation and function. Gene silencing of GCR8, Dicer, or Ago2 by small interfering RNAs revealed global inhibition of osteoclast transcription factor expression and function, decreased osteoclastogenesis, and decreased bone resorption *in vitro. In vivo,* CD11b (+) -cre/Dicer-null mice had mild osteopetrosis caused by decreased osteoclast number and bone resorption. These results provide hints as to novel molecular mechanisms controlling osteoclast differentiation and function by the microRNA system and specifically by miR-223, which regulates the Nuclear factor 1 A-type (NFI-A) and M-CSFR levels (Sugatani et al., 2009). BMP-4-induced osteoblastic differentiation of bone marrow-derived ST2 stromal cells was promoted and repressed after transfection of sense and antisense miR-210, respectively. This observation demonstrated that miR-210 acts as a positive regulator of osteoblastic differentiation by inhibiting the TGF-beta/activin signaling pathway through inhibition of AcvR1b (Mizuno et al., 2009). Mesenchymal stem cells are excellent candidates for cell-based therapeutic strategies to regenerate injured tissue. Study by Schoolmeesters et al. (2009), employed a library of microRNA inhibitors to evaluate the role of microRNAs in early osteogenic differentiation of human Mesenchymal stem cells. It was discovered that miR-148b, -27a and -489 are essential for the regulation of osteogenesis: miR-27a and miR-489 down-regulate while miR-148b up-regulates osteoblast differentiation. Modulation of these microRNAs induces osteogenesis in the absence of other external differentiation cues and thus restores osteogenic potential of high passage number in human Mesenchymal stem cells. MiR-29b promotes osteogenesis by directly down-regulating the known inhibitors of osteoblast differentiation, HDAC4, TGFbeta3, ACVR2A (Activin receptor type-2A gene), CTNNBIP1 (catenin, beta interacting protein 1), and DUSP2 proteins through binding to target 3'-UTR sequences in their mRNAs. Thus, miR-29b is a key regulator of development of the osteoblast phenotype by targeting anti-osteogenic factors and modulating bone extracellular matrix proteins (Li et al., 2009). They also identify a new microRNA (miR-2861) in primary mouse osteoblasts that promotes osteoblast differentiation by repressing histone deacetylase 5 (HDAC5) expressions at the post-transcriptional level. HDAC5, an enhancer of runt-related transcription factor 2 (Runx2) degradation. *In vivo* silencing of miR-2861 in mice reduced Runx2 protein expression, inhibited bone formation and decreased bone mass. Importantly, miR-2861 was found to be conserved in humans, and a homozygous mutation in pre-miR-2861 that blocked expression of miR-2861 was shown to cause primary osteoporosis. Later on Hu et al. (2011) also found the same function of miR-2861. Besides that, they found another new microRNA (miR-3960) that played a regulatory role in osteoblast differentiation through a regulatory feedback loop with miR-2861. Homeobox A2 (Hoxa2), a repressor of Runx2 expression, was confirmed to be a target of miR-3960. It has been reported that miR-3960 and miR-2861, transcribed together from the same microRNA polycistron, both function in osteoblast differentiation through a novel Runx2/miR-3960/miR-2861 regulatory feedback loop (Table 2). These findings provide new insights into the roles of microRNAs in osteoblast differentiation (Hu et al., 2011). In an early study by Sugatani et al., (2007), it was found that miR-223 plays an essential role during osteoclast differentiation and miR-223 might be a viable therapeutic target for a range of bone metabolic disorders with excess osteoclast activity. Later on they demonstrated that Gene silencing of Di George Syndrome Critical Region 8 (DGCR8), Dicer, or; Argonaute protein (Ago2) by small interfering RNA revealed global inhibition of osteoclast transcription factor expression and function, decreased osteoclastogenesis and decreased bone resorption *in vitro* and *in vivo*. A novel mechanism mediating these results was demonstrated and was shown that PU.1, microRNA-223, NFI-A and the macrophage colony-stimulating factor receptor (M-CSFR) are closely linked through a positive feedback loop (Sugatani et al., 2009). Connexin 43 (Cx43), a major gap junction protein in osteoblasts, has been shown as a target of miR-206, and restoration of Cx43 expression in

miR-206-expressing osteoblasts rescued them from the inhibitory effect of miR-206 on osteoblast differentiation (Inose et al., 2009). Canonical Wnt signaling is particularly important for maintenance of bone mass in humans. The miR-29 and Wnt signaling are involved in a regulatory circuit that can modulate osteoblast differentiation. Specifically, canonical Wnt signaling induces miR-29a transcription. The subsequent down-regulation of key Wnt signaling antagonists, Dkk1, Kremen2, and sFRP2, by miR-29a potentiates Wnt signaling, contributing to a gene expression program important for osteoblast differentiation (Kapinas et al., 2010). Intrinsically, Dicer deficiency in osteoclasts suppressed the levels of TRAP positive multinucleated cell development in culture and also reduced NFATc1 and TRAP gene expression. MicroRNA analysis indicated that expression of miR-155 was suppressed by RANKL (Receptor activator of nuclear factor kappa-B ligand) treatment in Dicer deficient cells. Dicer deficiency in osteoclasts suppressed osteoblastic activity *in vivo* including mineral apposition rate (MAR) and bone formation rate (BFR) and also suppressed expression of genes encoding type I collagen, osteocalcin, Runx2, and Efnb2 *in vivo*. Dicer deficiency in osteoclasts increased the levels of bone mass. Thus, indicating that the Dicer deficiency-induced osteoclastic suppression was dominant over Dicer deficiency-induced osteoblastic suppression (Mizoguchi et al., 2010). Unrestricted somatic stem cells (USSCs) have been identified in human umbilical cord blood and have been shown to differentiate into lineages representing all 3 germ layers. To characterize microRNAs that may regulate osteogenic differentiation of USSCs, the expression analysis was carried out for 157 microRNAs using quantitative RT-PCR before and after osteogenic induction and suggested that hsa-miR-135b may control osteoblastic differentiation of USSCs by regulating expression of bone-related genes (Schaap-Oziemlak et al., 2010). Taipaleenmaki et al. (2011) in a review highlighted the current knowledge of microRNA biology and their role in bone formation and discuss their potential use in future therapeutic applications for metabolic bone diseases.

Table 2. Summary of microRNAs involment in bone remodelling cells and in bone pathogenesis

microRNA in Osteoclast proliferation and differentiation	microRNA in Osteoblast proliferation and differentiation	microRNA in Chondrocytes/ Arthritis	microRNA in Osteosarcoma
miR-223, 130a,155, 21,302c.	miR-125b, 199a, 26a, 29b, 206, 133, 135, 29a, 141, 200, 210, 29,378, 2861,3960, 370, 34s, 34. **Mineralisation-** 93, 135b	miR-18a, 199a, 146a, 222, 140, 27b,	miR-183, 181a, 181b, 181c, 20a, 125b, 382,369-3p, 544, 134.

Receptor activator of nuclear factor B ligand (RANKL)-induced osteoclastogenesis is mediated by miR-21. MiR-21 was identified as signature of RANKL-induced osteoclastogenesis that down-regulates programmed cell death 4 (PDCD4) protein levels. Diminished PDCD4 removes a repression from c-Fos, a critical transcription factor for osteoclastogenesis and osteoclast-specific downstream target genes. In addition, RANKL-induced c-Fos up-regulates miR-21 gene expression. Thus, these studies (Hu et al., 2011; Sugatani et al., 2011) provide a new molecular mechanism, including a positive feedback loop of c-Fos/miR-21/PDCD4, regulating osteoclastogenesis. Although various microRNAs regulate cell proliferation and differentiation, few microRNAs have been reported to play a key role in the regulation of osteoclast differentiation. Xia et al. (2011), in a short review, summarized the biology and functional mechanisms of microRNAs in osteoclastogenesis

along with their profiling, function, and target prediction. In the same year, Sugatani et al. (2011) also highlight the role of microRNAs in osteoclastogenesis and provide a new molecular mechanism, including a positive feedback loop of c-Fos/miR-21/PDCD4, regulating osteoclastogenesis. Several nanomaterials induced enhanced mineralization (increased numbers and larger areas of mineral nests) in MC3T3-E1 bone cells, with the highest response being induced by silver nanoparticles (AgNPs). AgNPs altered the microRNA expression resulting in specific gene expression associated with bone formation (Mahmood et al., 2011). Chang et al. (2011) in a study demonstrate five microRNAs enriched in WJ-MSCs, including miR-345, miR-106a, miR-17-5p, miR-20a and miR-20b. Another 11 microRNAs (miR-206, miR-34a, miR-374, miR-424, miR-100, miR-101, miR-323, miR-368, miR-137, miR-138 and miR-377) were abundantly expressed in transdifferentiated neuronal progenitors. Among these microRNAs, miR-34a and miR-206 were the only 2 microRNAs, which have been linked to BM-MSC neurogenesis. Over expressing miR-34a in cells suppressed the expression of 136 neuronal progenitor genes, which all possess putative miR-34a binding sites. Bone development is dynamically regulated by homeostasis, in which there is a balance between adipocytes and osteoblasts. Preliminary data indicated that miR-637 suppressed the growth of hMSCs and induced S-phase arrest. Expression of miR-637 was increased during adipocyte differentiation (AD), whereas it was decreased during osteoblast differentiation (OS), which suggests miR-637 could act as a mediator of adipo-osteogenic differentiation. Osterix (Osx), a significant transcription factor of osteoblasts, was shown to be a direct target of miR-637, which significantly enhanced AD and suppressed OS in hMSCs through direct suppression of Osx expression (Zhang et al., 2011). The Periodontal ligament stem cells (PDLSCs) were treated with ibandronate promotes its proliferation and enhanced the expression of alkaline phosphatase (ALP), type I collagen (COL-1), osteoprotegerin (OPG), osteocalcin (OCN), and Runx2. The expression of microRNAs, including miR-18a, miR-133a, miR-141 and miR-19a, was significantly altered in the PDLSCs cultured with ibandronate show regulation of the expression of diverse bone formation-related genes via microRNAs (Zhou et al., 2011). Overexpression of miR-93 in cultured primary mouse osteoblasts attenuates osteoblast mineralization. Sp7 transcription factor 7 (Sp7, Osterix), a zinc finger transcription factor and critical regulator of osteoblast mineralization, was found inversely correlated with miR-93. Thus, miR-93 was an important regulator in osteoblast mineralization through a novel miR-93/Sp7 regulatory feedback loop (Yang et al., 2012). Recent studies have shown that miR-9 plays a regulatory role in the development and differentiation of stem cells and neural precursor cells. It has been found that miR-9 is able to promote the differentiation of bone marrow mesenchymal stem cells (MSCs). It is established that Zfp521 could induce neural conversion of embryonic stem cells. In a study, Han et al. (2012), found that the expression of Zfp521 declined with the neural differentiation of MSCs, and miR-9 could promote the neural differentiation via targeting Zfp521. MiR-96, miR-124 and miR-199a, were differentially expressed during osteogenic, adipogenic and chondrogenic induction of human bone marrow-derived MSCs. MiR-96 expression was increased during osteogenesis and adipogenesis, but not during chondrogenesis. MiR-124 was exclusively expressed in adipocytes, whereas miR-199a was upregulated in osteoblasts and chondrocytes. Furthermore, miR-96, miR-124 and miR-199a regulated the expression of genes important for hMSC differentiation, such as aggrecan, transcription factor SOX9 and fatty acid binding protein 4 (FABP4). Thus, modulation of miR-96, miR-124 and miR-199a expression may thus be useful in specific targeting of hMSC differentiation (Laine et al., 2012). Also in a review article in same year, Laine et al. summarizes the current insights into microRNA-mediated regulation of bone marrow stem/progenitor cell maintenance and differentiation. The FoxO family is widely accepted to play an important role in protecting diverse cells from reactive oxygen species (ROS). Activation of FoxO1, the main FoxO in bone, stimulates

proliferation and differentiation as well as inhibits apoptosis of osteoblast lineage cells. Kim et al. (2012) identified additional crucial microRNA, miR-182 in zebrafish, which regulates osteoblastogenesis by repressing FoxO1 and thereby negatively affecting osteogenesis and impaired bone formation. Transient modulation of multiple osteo-miRs (such as miR-199b, 1274a, 30b) with common targets (such as BMPR, TCFs, SMADs) which acts as mediators of osteogenic pathways; including cell-cell interactions, WNT and TGF-beta pathways, suggests a mechanism for rapid induction of the osteogenesis as an anti-microRNA therapy. Thus, the study has identified the microRNA signature, which regulates the osteogenesis mechanism in unrestricted somatic stem cell (Bakhshandeh et al., 2012). Lian et al. (2012) in a review highlighted the emerging concept of bone-regulating microRNAs. The evidence for which, has been gathered largely from *in vivo* mouse models and in vitro studies in human and mouse skeletal cell populations. It has been indicated that microRNAs are closely related to osteogenesis. Previous data suggested that miR-30 family members might be important regulators during the biomineralization process. However, whether and how they modulate osteogenic differentiation has not been explored. In a study, Wu et al. (2012) demonstrated that miR-30 family members negatively regulate BMP-2-induced osteoblast differentiation by targeting Smad1 and Runx2 in mouse bone marrow mesenchymal stem cells. Dong et al. (2012) review the recent research progress on the regulation of microRNAs in bone biology, with a particular focus on the microRNA-mediated control mechanisms of bone and cartilage formation.

6.3 MicroRNAs in Bone Tumor

Osteosarcoma (OS) is a cancerous (malignant) bone tumor that usually develops during the period of rapid growth that occurs in adolescence. Most of the work will be done in microRNA in Osteosarcoma (OS) in last two years. The ability of OS cells to form lung metastases has been inversely correlated to cell surface Fas expression. Here, Huang et al. (2011) show that microRNA plays a role in the downregulation of Fas expression in OS. Expression levels of several members of the miR-17-92 cluster including miR-20a and miR-19a were found to be higher in metastatic low-Fas-expressing LM7 cells than in the parental non metastatic high-Fas-expressing SAOS-2 cells. Abnormally expressed miR-125b was found to play a fundamental role in several types of cancer; however, whether miR-125b participates in regulating the initiation and progress of OS still remains unclear. Here they demonstrate that miR-125b is frequently down-regulated in OS samples and human OS cell lines. Interestingly, Liu et al. (2011) discovered that the expression of miR-125b is regulated by signal transducer and activator of transcription 3 (STAT3) at transcription level (Table 2). STAT3 binds to the promoter region of miR-125b in vitro and serves as a transactivator. These findings point to an important role in the molecular etiology of OS and suggest that miR-125b is a potential target in the treatment of OS. MiR-34c is significantly induced by BMP2 during osteoblast differentiation. In osteoblasts, miR-34c targets multiple components of the Notch signaling pathway, including Notch1, Notch2 and Jag1 in a direct manner. MiR-34 influences osteoclast differentiation in a non-cell-autonomous fashion. Therefore, by understanding the functional interaction of miR-34 and Notch signaling in normal bone development and in bone cancer could potentially lead to therapies modulating miR-34 signaling (Bae et al., 2012). Kobayashi et al., (2012) recently summarize the current understanding of the roles that microRNAs play in OS and highlight their potential as biomarkers or therapeutic targets. In another study it was found that Down-Regulation of miR-183 Promotes Migration and Invasion of OS by Targeting Ezrin (Zhu et al., 2012). MicroRNA signature also reflects the pathogenesis of OS from surgically procured samples from human patients. The signature includes high expression of miR-181a,miR-181b, and miR-181c as well as reduced expression of miR-16, miR-29b, and miR-142-5p.Jones et al.

(2012) also demonstrate that miR-181b and miR-29b exhibit restricted expression to distinct cell populations in the tumor tissue. Further, higher expression of miR-27a and miR-181c in pre-treatment biopsy samples characterized patients who developed clinical metastatic disease. Thus, their findings establish a microRNA signature associated with pathogenesis of OS as well as critical pre-treatment biomarkers of metastasis and responsiveness to therapy. Although several genetic predisposing conditions have been associated with OS the understanding of its molecular etiology is limited. Here, Thayanithy et al. (2012) show that microRNAs at the chr.14q32 locus are significantly downregulated in OS compared to normal bone tissues.

Several specific miRNAs involved in OS have been shown to be upregulated or downregulated in OS, as described above. These results suggest that miRNA-based therapeutic strategies to restore or inhibit the expression of miRNAs can serve as a novel therapeutic option for OS. Due to their tissue specificities and critical roles in various biological processes, various studies indicate that miRNAs have the potential to be promising diagnostic biomarkers, as well as therapeutic targets in OS. Moreover, miRNAs are detectable even in the sera of patients, leading to additional potential clinical applications. There has already been significant progress in the basic knowledge of sarcoma, which has uncovered the basis of many multiple biological processes. However, the research on the correlations of miRNAs with OS has just begun. Although miRNAs will undoubtedly contribute to the advancement of future clinical therapeutic applications for OS, further investigations and the establishment of ideal *in vivo* delivery systems will be essential.

6.4 MicroRNAs in Arthritis

"Arthritis" is a common musculoskeletal condition in which several microRNAs plays an important role in them. MiR-146a is a negative regulator of immune and inflammatory responses and is strongly expressed in rheumatoid arthritis (RA) synovial and peripheral blood mononuclear cells (PBMCs). MiR-146a expression inhibits osteoclastogenesis and administration of miR-146a prevents joint destruction in mice with 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) (Nakasa et al., 2011). MiR-223 is upregulated in rheumatoid arthritis (RA) patients and is involved in osteoclastogenesis that contributes to erosive disease and first to demonstrate that lentivirus-mediated silencing of miR-223 can reduce disease severity of experimental arthritis in mice (Li et al., 2012). Coordinated actions of various regulators including morphogens are required for chondrogenesis and maintenance of articular cartilage function MicroRNAs are an integral part of the regulatory network in chondrocyte differentiation and cartilage function. Hong et al. (2012) in a review demonstrated the progress in microRNA expression and target genes in cartilage differentiation, homeostasis, and in the pathobiology of osteoarthritis (OA). Recent findings show that the expression of miR-140, which is specifically expressed in chondrocytes, is reduced in osteoarthritic chondrocytes. Furthermore, knockdown of miR-140 in mice chondrocytes promotes arthritis in mice. Thus, microRNAs should be critical factors for cartilage development and homeostasis (Asahara, 2012). In a study Koichi et al. (2010) found significant different expression of miR-16, miR-146a miR-155 and miR-223 in synovial fluid of rheumatoid joint than those of OA. Ratio of synovial fluid miRNAs to plasma miRNAs, including miR-16 and miR-146a, significantly correlated with tender joint counts and 28-joint Disease Activity Score. As plasma microRNAs had distinct patterns from synovial fluid microRNAs, which appeared to originate from synovial tissue. Plasma miR-132 expression

was well different in healthy controls than that in patients with RA or OA, and correlated with different expression of synovial fluid microRNAs in RA and OA. Furthermore, plasma microRNAs correlated with the disease activities of RA. Thus, synovial fluid and plasma microRNAs have potential as diagnostic biomarkers for RA and OA and as a tool for the analysis of their pathogenesis.

6.5 MicroRNAs in Osteoporosis

Osteoporosis is a skeletal disease that is characterized by compromised bone strength predisposing a person to an increased risk of fracture. Leptin-serotonin-sympathetic nervous system pathway plays a major role as evident from the fact that beta-blockers reduced the risk of osteoporotic fractures in human. Neuronal and microRNA-dependent pathways are promising candidates for new therapeutic applications for osteoporosis treatment (Takeda et al., 2011). MicroRNAs have important regulatory role in osteogenesis and cartilage growth and regeneration, but the definite mechanisms have not been clear yet (Zeng et al., 2011). Kapinas et al. in a review will highlight the current understanding of microRNA biogenesis and mechanisms of action and summarize recent work on the role of microRNAs, including the miR-29 family, in bone remodeling (Kapinas et al., 2011). Molecular mechanisms that regulate human stromal (mesenchymal) stem cell (hMSC) differentiation into osteogenic lineage are important for the development of anabolic therapies for treatment of osteoporosis. It has been showed that miR-138 modulates osteogenic differentiation of hMSCs. MicroRNA array profiling and further validation by quantitative RT-PCR (qRT-PCR) revealed that miR-138 was down-regulated during osteoblast differentiation of hMSCs. Overexpression of miR-138 inhibits osteoblast differentiation of hMSCs in vitro, whereas inhibition of miR-138 function by antimiR-138 promoted expression of osteoblast-specific genes, alkaline phosphatase (ALP) activity, and matrix mineralization. Furthermore, overexpression of miR-138 reduced ectopic bone formation *in vivo* by 85%, and conversely, *in vivo* bone formation was enhanced by 60% when miR-138 was antagonized (Eskildsen et al., 2011).

MicroRNAs regulate posttranscriptional gene expression usually by binding to 3'-untranslated regions (3'-UTRs) of target mRNAs. Hence genetic polymorphisms on 3'-UTRs of mRNAs may alter binding affinity between microRNAs target 3'-UTRs, thereby altering translational regulation of target mRNAs and/or degradation of mRNAs, leading to differential protein expression of target genes. Lei et al. find this differential expression in the fibroblast growth factor 2 (FGF2) gene between subjects with high versus low BMD concluded that these three polymorphisms of the FGF2 gene may contribute to susceptibility to osteoporosis, most likely through their effects on altered binding affinity for specific microRNAs (Lei et al., 2011). Wharton's jelly matrix of human umbilical cord (WJ-MSCs) are able to transdifferentiate into neuronal lineage cells both *in vitro* and *in vivo* and therefore hold the potential to treat neural disorders such as stroke or Parkinson's disease. In bone marrow MSCs, miR-130a and miR-206 have been show to regulate the synthesis of neurotransmitter substance P in human mesenchymal stem cell-derived neuronal cells. However, how neuronal differentiation is controlled in WJ-MSC remains unclear (Taipaleenmaki et al., 2012). Wang et al. performed a bioinformatic target gene analysis and found negative correlations between miR-133a and three potential osteoclast-related target genes, CXCL11, CXCR3 and SLC39A1. So, they suggested that miR-133a in circulating monocytes is a potential biomarker for postmenopausal osteoporosis (Wang et al., 2012). Liao et al. (2012) in ovarectomised mice, found a serials of differentially expressed microRNAs in bone marrow-derived mesenchymal stem cells during osteoporosis and their function in stem cell differentiation.

6.6 MicroRNAs in Bone Metastasis

Development of bone metastases is dependent on the cancer cell-bone cell interactions in the bone microenvironment. Transforming growth factor (TGF-) is released from bone during osteoclastic bone resorption and induces production of osteolytic factors, such as interleukin 11 (IL-11), as in breast cancer cells. IL-11 in turn increases osteolysis by stimulating osteoclast function, launching a vicious cycle of cancer growth and bone destruction. Pollari et al. (2012) in a study showed that miR-204, miR-211, and miR-379 directly target IL11 by binding it to 3' UTR. MiR-379 also inhibits Smad2/3/4-mediated transcriptional activity. Gene expression analysis of miR-204 and miR-379-transfected cells indicated that these microRNAs downregulated the expression of several genes involved in TGF- signaling, including prostaglandin-endoperoxide synthase 2 (PTGS2). They found a significant correlation between the genes downregulated by miR-379 and a set of genes upregulated in basal subtype of breast cancer. Menin is the product of the MEN1 oncosuppressor gene, responsible for multiple endocrine neoplasia type 1 syndrome. Menin expression modulates mesenchymal cell commitment to the myogenic or osteogenic lineages. The miR-26a modulates the expression of SMAD1 protein during the osteoblastic differentiation of human adipose tissue-derived stem cells (hADSCs). This study evidence gives a direct interaction between menin transcription factor and microRNA interaction that seems to play a pivotal role during the hADSCs osteogenesis. Thus this observation suggesting a novel target for bone disease RNA-based therapy (Luzi et al., 2012). MiR-370 regulates the expression of bone morphogenetic protein-2 (BMP-2) and V-ets Erythroblastosis Virus E26 Oncogene Homolog 1 (Ets1) in BMP-2-stimulated murine pre-osteoblast MC3T3-E1 cell differentiation. The enforced expression of mature miR-370 in MC3T3-E1 cells or primary osteoblast cells remarkably attenuated BMP-2-induced pre-osteoblast differentiation. Itoh et al. (2012) hypothesized a BMP-2-Ets1-PTHrP feed-forward loop regulatory mechanism.

7. CONCLUSIONS

The discovery of microRNAs has led to deeper insights into the regulation mechanism of gene expression and its complexity. Now, several studies have demonstrated the importance of microRNAs in bone remodeling via control of osteoblast and osteoclast proliferation, differentiation and as well as mineralization. An in-depth understanding of the roles of these regulatory RNAs in the skeleton will be vital for the evolution of new therapeutics for bone loss of various origins, their pathogenesis and perhaps may also facilitate bone remodelling. Although current therapies can be effective in preventing bone loss, there are patients who are refractory to these treatments. The inhibition or over-expression of certain microRNAs in a tissue-specific manner has shown some promise in the treatment of many life threatening diseases like tumors in animal models. Two main benefits of microRNA-based therapeutics has been shown; First it is relatively cost effective as compared to peptide-based therapy and second it can be targeted to specific tissues using relatively non-toxic delivery vehicles. Beside its therapeutics role, currently some of the microRNAs are being considered as biomarkers in various pathological conditions because of fact that their significant levels have been found in extracellular human body fluids including blood plasma, urine, saliva and semen. Though much of the recent work has been done to establish the role of these regulatory RNAs in bone metabolism, we observe that there is a need to detect novel biomarkers for early detection of bone disease. Clinical trials based on the therapeutic role of micro-RNA in bone remodeling, fracture repair, and many bone-related diseases would open up new horizons of clinical practice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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