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MicroRNAs as Next Generation Therapeutics in Osteoporosis

Taruneet Kaur, Rajeev Kapila and Suman Kapila

Abstract

Bone is an active tissue that works as a tissue and an organ as well. It is constituted of cells and blood vessels by nearly 10% of its volume, while the rest 90% is majorly contributed by extracellular portion. Bone is a living structure stably undertaking continual remodeling between bone formation and bone resorption, where bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts) exhibit a crucial role. The differentiation process of osteoblasts and osteoclasts takes place in a balanced manner under normal conditions. This intricate balance is chiefly sustained by biochemical signaling cascades, facilitating accurate bone homeostasis in the body. Loss of balance/misregulated signaling in the bone development or disruption may lead to pathological conditions such as osteoporosis, arthritis, etc. Among several regulators for bone-signaling pathways, microRNAs have appeared as an imperative control of gene expression at the level of post-transcription while addressing the genes that control bone remodeling with appropriate responses in the pathogenesis and perhaps the management of bone diseases. Further, microRNAs control the proliferation and differentiation of osteoblasts and osteoclasts, which finally influence the bone formation. Hence, there is a great possibility in exploiting microRNAs as putative therapeutic targets for the medical relief of bone associated disorders, including osteoporosis.

Keywords: bone formation, signaling pathways, osteoporosis, microRNAs, therapeutics

1. Introduction

Bone is a tough and dynamic tissue that provides shape to our body while protecting the organs [1]. Bone formation is chiefly regulated by the precise biochemical signaling pathways that maintain the action of the bone cells viz. osteoblasts and osteoclasts [2]. These bone cells act in a balanced and stable fashion in the normal functioning of the bone. Disruptions in this intricate balance results in absurd bone functions with the consequent occurrence of bone associated disorders. Osteoporosis is one such well-recognized bone disease that is patented by the decreased bone mineral density and loss of connectivity in the bone trabeculae [3]. Osteoporosis has been documented to elicit approximately 8.9 million fractures annually, targeting around 200 million osteoporotic women across the globe [4, 5]. As per the International Osteoporosis Foundation, osteoporosis results in 1.5 million fractures per year in the USA while in Europe, more than 3.5 million osteoporotic fractures have been reported each year [6, 7]. Besides, in

India approximately 61 million people are stated as diseased osteoporotic patients [8, 9]. Therefore, management of osteoporosis is the urgent need of the hour for providing relief to the masses and hence refining the quality of life. Apart from numerous therapeutic measures available for the treatment, osteoporosis is still largely undertreated and seeks improved strategies that are associated with fewer side effects.

MicroRNA (miRNA) antagonism might be a new therapeutic approach for the check of osteoporosis. miRNAs are small RNAs (21–23 nucleotides) that act as post-transcriptional regulators of gene expression [10]. In a broader sense, miRNAs execute their functions either by degrading the gene (mRNAs) or by repressing the translation of the protein for the respective gene [11]. miRNA mediated targeting of expression for the genes that are involved in bone remodeling and regeneration is a novel and specific mode of therapeutic strategy. Various miRNAs control the proliferation and differentiation potential of osteoblasts and osteoclasts that ultimately regulates the bone formation [12]. There are numerous studies that report the prominence of miRNA functions and miRNA-based antagonism in the maintenance of bone homeostasis [13]. While acting on the mRNAs, miRNAs can stimulate as well inhibit the activity of osteoblasts and osteoclasts in the bone remodeling [14]. miRNAs are able to obstruct the excessive bone loss during osteoporosis and encourages the bone formation [15]. In short, miRNAs are anticipated to serve as putative gene therapy targets for the treatment of bone-related injuries [16]. Hence the present chapter details the role and efficacy of miRNA in the management of osteoporosis. Finally, we describe the mechanism by which miRNAs can regulate the gene expression in bone formation and resorption.

2. Bone

Bone is an active connective tissue that behaves like an organ as well [1]. The most fundamental function of the bone is to offer support and shape to the body [17]. Other than that, bone also serves endocrine functions and assists in hematopoiesis [18]. Broadly, the structure of bone is categorized into two types, namely cancellous or cortical bone. Cortical/compact type comprises of 80% of the bone skeleton, whereas cancellous/spongy makes up to 20% [19]. Cortical bone forms the dense outer linings of the strong bones, and spongy bone is present at the ends of the long bones [20]. Bone is associated with dynamic character and undertakes continual remodeling, wherein the aged bone is resorbed, and new bone is continually ossified [21]. The bone resorptive episode takes around 10 days to complete, whereas the formation of bone persists for a period of 3 months [22].

2.1 Components of bone

Two vital components of the bone composition are matrix and cells [23]. Matrix is further consists of organic part (30%) and inorganic part/minerals (70%) [23]. Around 90% of the bone organic matrix is made of collagen type 1 and rest 10% includes proteins such as osteocalcin, osteopontin, osteonectin, etc. [19]. In addition, bone is a storehouse for minerals, especially calcium and phosphorous, that makes the hydroxyapatite element of the bone. Hydroxyapatite provides framework and strength to the bone. Furthermore, bone is comprised

of four basic cell types, viz. osteoblasts, osteoclasts, osteocytes and bone lining cells [24]. Osteoblasts are bone-forming cells that arise from mesenchymal stem cells. Several growth (FGF) and transcription factors (Runx2, Osterix) are responsible for the differentiation of mesenchymal cells to the osteoblastic lineage [25]. Osteoclasts are bone-resorbing cells that originate from hematopoietic monocyte-macrophage lineage, which differentiates via the assistance of the receptor activator of nuclear factor-κB ligand (RANK ligand) and Macrophage colony-stimulating factor (M-CSF) [26]. Osteoclasts are the biggest (in size) of all other cell types of the bone. Osteocytes constitute 95% of the cells in the mature skeleton [1]. These are the mineralized differentiated osteoblast cells that regulate the process of bone remodeling. Bone lining cells are a type of flat osteoblastic cells that sheet the quiescent bone surfaces [27]. They favor to safeguard the bone, maintain the bone fluids and form a barrier between the bone and bone marrow.

3. Physiological bone regeneration

Bone modeling is a specialized process wherein old bones are removed from one location and replaced by new bone at a distinct location. This process defines the ultimate shape and size of the skeleton [28]. While bone remodeling is a characteristic process in the mature skeleton that is marked by constant bone restoration via a frequent exchange of aged bone with the fresh one at the same site. The process results in the comprehensive regeneration of mature skeleton in an adult every 10 years [29]. The body tries to sustain the balance between bone formation and elimination during the process of bone remodeling. It takes place in discrete sites called basic multicellular units (BMU). The process initiates by activation phase where an initiating signal (e.g., mechanical strain on the bone, fracture healing, etc.) flags the requirement of the remodeling process [30]. After the activation, the commencement of the resorptive phase occurs, wherein osteoclasts depletes bone by proteolytic degradation and acidification. Osteoblasts travel to the eroded space and begin the ossification after the stimulation of transcription factors that encourages the bone formation [31]. Ossified bone is subsequently mineralized and eventually remodeling cycle ends.

4. Biochemical signaling pathways that regulate the bone formation

Bone formation is controlled by numerous elements including transcription factors, hormones, growth factors, oxidative processes, mechanical loading, stress, bone fractures and aging [32]. Osteoblasts and osteoclasts are able to read these external stimulants and propagate the biochemical signals via various signaling cascades. The biological response of the selected signaling pathways results either in bone formation or disruption. Some of the crucial pathways operating in the osteoblasts and osteoclasts are described as follows:

4.1 Wnt/β-catenin pathway

The Wnt signaling pathway has an enormous vital role in the bone development and maintenance of bone homeostasis [33]. Wnt is a secreted protein ligand that

binds to a receptor complex of Frizzled (Fz) and low-density lipoprotein receptor-related proteins (LRP). There are two modes of functioning for the Wnt proteins, i.e., canonical and non-canonical pathways, wherein the canonical pathway has a more specific role to play in the bone development. In the canonical pathway (**Figure 1**), the interaction of Wnt to the receptor complex hinders the functioning of axin, gly-cogen synthase kinase 3β (GSK-3β) and adenomatous polyposis coli (APC) protein. This primes the accumulation of β-catenin in the cytoplasm, further β-catenin travels down to the nucleus and ultimately stimulates lymphoid-enhancer-binding factor/T-cell-specific transcription factors (LEF/TCF). This results in the transcriptional activation for the genes that participate in bone formation and regeneration. While the absence of Wnt signal leads to phosphorylation of the cytosolic β-catenin and its subsequent ubiquitin-mediated degradation [34]. The degradation of the β-catenin finally turns off the downstream activation of the osteogenic genes. Accurate Wnt signaling is a pre-requisite for adequate bone mass in the body while mutation of the Wnt signaling components results in fractures and bone injuries [35].

4.2 BMP-Smad pathway

Bone morphogenetic protein (BMP) holds a well-known and fundamental role in the bone development [36]. BMP signaling is initiated through the interaction of BMPs with the BMP-receptors (type I and type II). This binding stimulates the
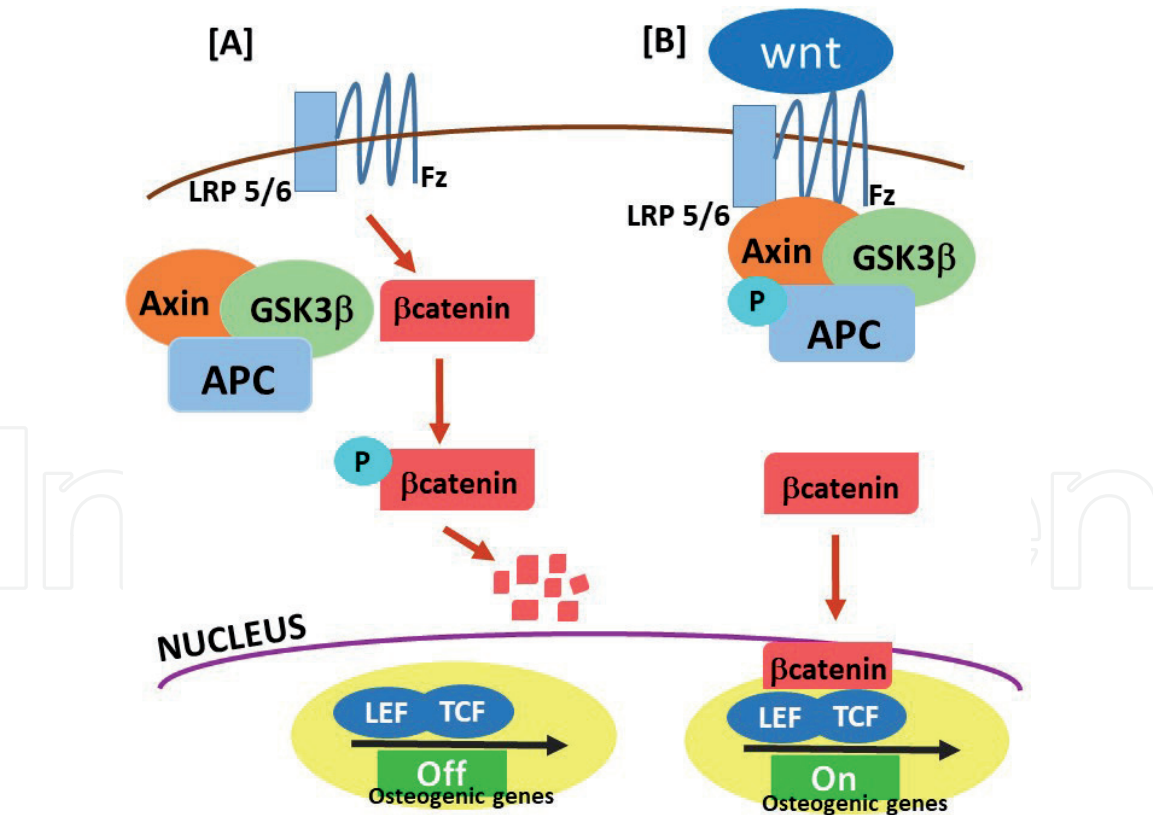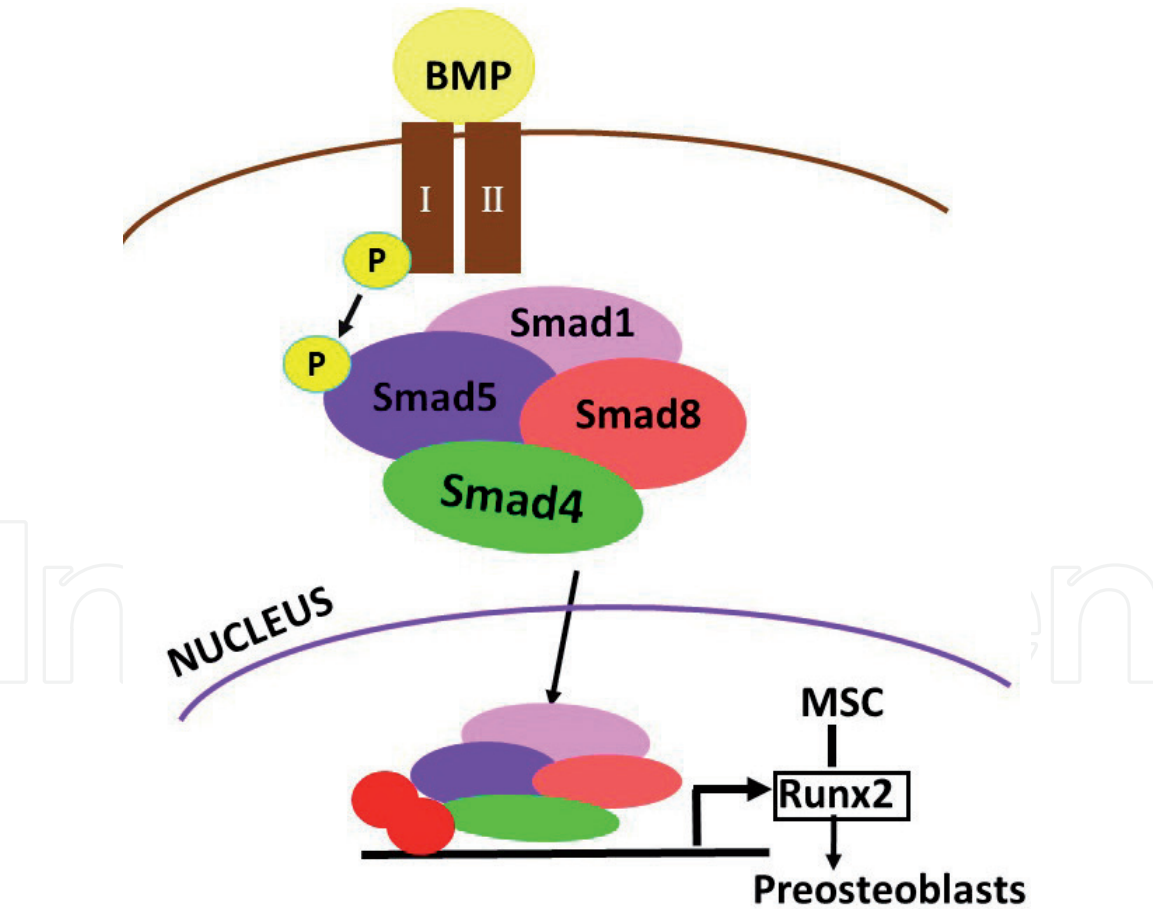

Figure 1.
Wnt/β-catenin (canonical) signaling pathway in the osteoblasts. [A] In the absence of Wnt protein, the degradation complex Axin, GSK-3β and APC protein phosphorylates the β-catenin and results in the ubiquitin-mediated degradation of β-catenin. This turns off the transcription machinery for the osteogenic genes and hence transcription for the genes involved in osteogenic differentiation is hampered resulting in defective bone formation. [B] Wnt ligand interacts with the receptor complex of Fz and LRP that restricted the action of Axin, GSK-3β, and APC protein and hence permitted the transport of β-catenin to the nucleus. β-catenin combines with LEF/TCF transcription factors in the nucleus, thereby potentiating the transcription for the osteogenic genes.

process of phosphorylation in the receptors that leads to recruitment and activation of Smad proteins, i.e., Smad 1, 5, 8 and Smad4. Smad proteins act as transcriptional regulators in the nucleus and ultimately induce the expression of the genes responsible for osteoblastogenesis (**Figure 2**) [37, 38]. There are a total of 14 members in the BMP family, out of which BMP-2,4,5,6,7 and 9 are reported to have high bone formation ability [37].

4.3 RANKL mediated signaling

RANKL-based pathway is an essential signaling cascade for the osteoclast differentiation. RANKL binds to its receptor RANK (present on the osteoclast precursors) and recruits TNF receptor-associated factor (TRAF) adaptor proteins to the conserved TRAF domain present at the cytoplasmic domain of the RANK [39, 40]. TRAF transduces the signal to downstream proteins viz. nuclear factor kappa B (NF-κB), extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and Nuclear Factor Of Activated T Cells 1 (NFATc1) (**Figure 3**) [41, 42]. NF-κB is an important regulator for the osteoclast differentiation. It is mainly responsible for the inflammation-based osteolysis and bone resorption [43].

Figure 2.
Bmp-Smad signaling pathway in the osteoblasts. BMP ligand binds to receptor complex viz. type I (BMPRI) and type II receptor (BMPRII). Type II receptor which is a serine/threonine kinase in nature phosphorylates and stimulates type I receptor. Upon activation, type I receptor causes phosphorylation of the downstream proteins—receptor activated Smads (R-Smads), Smad 1/5/8. Further, R-Smads complexes with co-Smad, Smad4 and hence the complex transports to the nucleus. In the nucleus, Smad complex interacts with coactivators and finally results in the transcription of osteogenic gene viz. Runx2. Runx2 is a master transcription factor for the bone development. It aids in the differentiation of mesenchymal stem cells (MSC) to the osteogenic lineage.
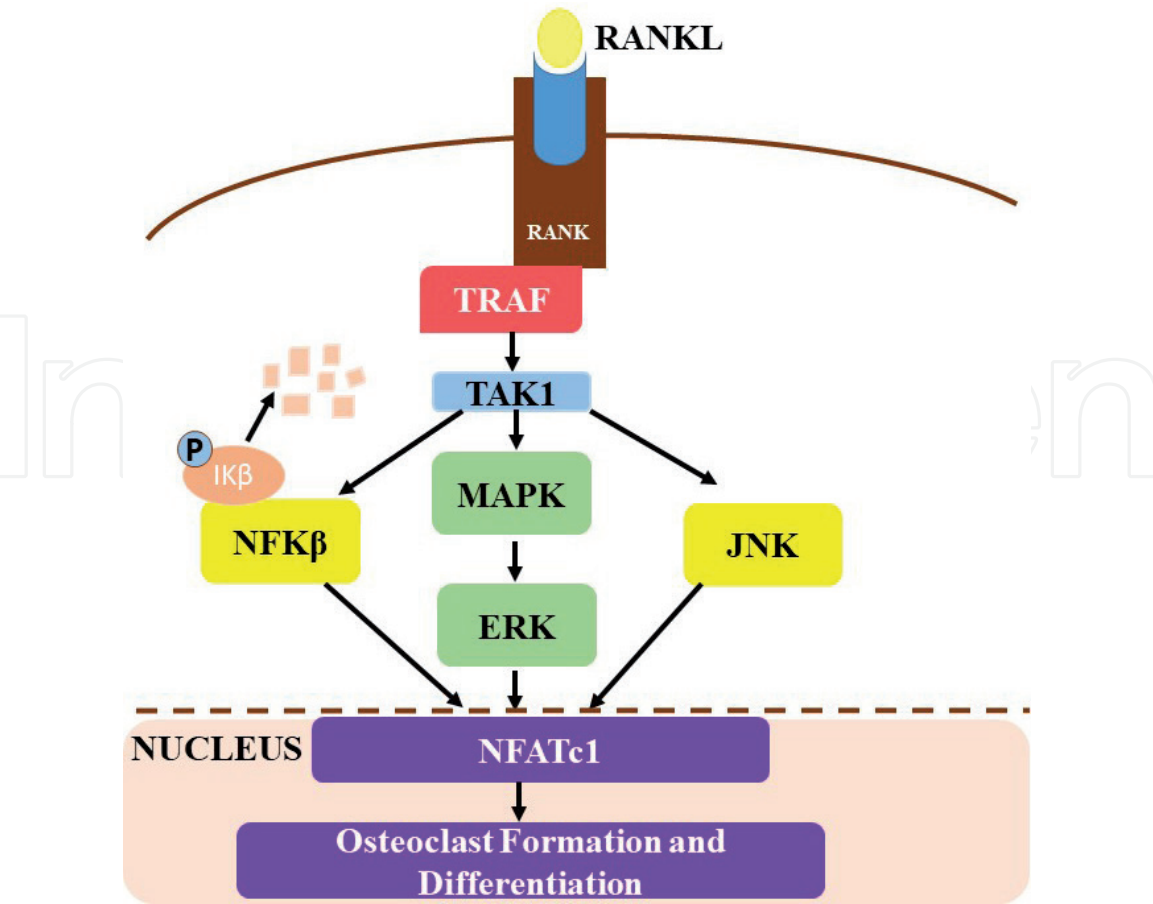
Figure 3.
RANKL-RANK signaling pathway in the osteoclasts. RANKL (present on the surface of osteoblasts)
interacts with RANK receptor (on the surface of osteoclasts). RANK does not possess any kinase activity and
hence recruits TRAF proteins to the cytoplasmic region of the receptor. This further transduces the signal to
downstream components and activates. (1) TAK1 (member of mitogen activated kinase family, MAPK),
promotes the ERK dependent activation of NFATc1. (2) NFK-β, after the phosphorylation based degradation
of inhibitor of NFK-β (IKβ). (3) Other downstream proteins viz. JNK that leads to transcriptional activation
of NFATc1 and additional factors resulting in the osteoclastogenesis.

5. Osteoporosis

Osteoporosis is regarded as a silent bone disorder. Though it is silent and associated with least symptoms, it seeks the most attention. It is reported that osteoporosis targets every third woman and every fifth man beyond 50 years of their ages [44]. Osteoporosis is a pathological condition in which bone mineral density is severely diminished with weakened bone microarchitecture [45]. Bones at the areas specific to hip, wrist and spine are highly vulnerable for the osteoporotic fractures. The occurrence ratio in female to male for the osteoporosis is 1:6 with 61% of fractures befalling in the women [46]. Besides, postmenopausal women have a greater tendency towards osteoporosis [47]. It is predicted that there would be around 6 million victims having osteoporosis by the year 2050 [48]. Osteoporosis is categorized into two major types mentioned as follows:

5.1 Primary osteoporosis

Primary osteoporosis is the most usual type of osteoporosis. It has two sub-categories: Type-I osteoporosis/postmenopausal osteoporosis, is a well-recognized bone issue in the postmenopausal women that is chiefly instigated by estrogen deficit due to menopause, while type-II osteoporosis/age-related osteoporosis is mainly caused as a result of aging in women and men both [49]. In Type-I primary

osteoporosis, bone loss hastens in the first 5 years of menopause and then slowly becomes constant. Estrogen executes the fundamental role in the bone development. It stimulates pro-collagen synthesis in the osteoblasts while acting against the bone resorbing cytokines [50, 51]. Estrogen is also capable of supporting osteoblast differentiation [52]. Moreover, estrogen deficiency induces the production of reactive oxygen species that results in increased osteoclast differentiation [53]. Thus, a shortage of estrogen after menopause is one prime cause of osteoporosis majorly in women. In the case of type II primary osteoporosis, age is the main cause that leads to fractures. With growing age, availability of minerals decreases, oxidants production increases while the body becomes less active and does not absorb calcium and vitamin D efficiently that overall thins out the density of bone and reduces the strength [54].

5.2 Secondary osteoporosis

Bone disorders which are secondary impediments of other health-related issues, e.g., adverse effects of drugs interventions, fluctuations in the cycle of physical activities, etc. are acknowledged under the category of secondary Osteoporosis [49]. Glucocorticosteroids and anticonvulsant-based interventions are majorly reported in the cases of secondary osteoporosis [55]. Several other disorders, e.g., endocrinopathies, which have the tendency to reduce the bone mass and interfere with normal bone formation, are also capable of inducing secondary osteoporosis. This form of osteoporosis is found in both pre/post- menopausal women and men [55].

6. Current therapeutic measures for osteoporosis

With recent advances in technology and knowledge, many therapeutic strategies are available for the management of osteoporosis (**Table 1**). Broadly, treatment measures against osteoporosis are classified under two classes: anti-resorptive and anabolic. Anti-resorptive agents work to reduce the rate of bone dissolution while anabolic agents attempt to boost bone formation and development.

Drug	Administration dose of the drug	Mechanism/effect	Harmful effects
Anti-resorptive strategies			
1. Bisphosphonates			
Alendronate	5 mg, 10 mg	Inhibits osteoclastogenesis by binding to minerals of the bone matrix	Severe joint and bone pain, serious allergic reactions, and osteonecrosis
Zoledronic acid	5 mg/ml	Diminishes osteoclast mediated bone disruption, also treats hypercalcemic conditions	Kidney-related issues, seizure, intense dizziness, and trouble while breathing
2. Estrogen modulators			
Raloxifene	60 mg	Mimics estrogen like effects in the bone that decreases bone resorption and enhances bone density	Risk of breast cancer, venous thromboembolism, and leg cramps

Drug	Administration dose of the drug	Mechanism/effect	Harmful effects
3. RANKL antagonist			
Denosumab	60 mg/ml	It is a human monoclonal antibody against RANKL that prevents the formation and maturation of osteoclasts	Shortness of breathing cycle, warm skin with pus, pain while urinating, and night sweats
Anabolic strategies			
1. Parathyroid hormone (PTH)			
Teriparatide	250 mcg/ml	It is a recombinant part of PTH, that stimulates the osteoblastogenesis with augmented bone mineral density	Heartbeat rate is increased, severe dizziness, allergies, itching and swelling of face, tongue, faintness, and osteosarcoma
Abaloparatide	2000 mcg/ml	Same as Teriparatide	Hypercalciuria, palpitations, and spinning sensation
2. Calcitonin	200 IU/ml	Encourages bone formation, reduces calcium levels in the plasma, increases net bone mass	Light-headed sensation, flushing, nausea, and vomiting

Table 1.
Different anti-osteoporotic therapeutic measures currently available in the market [56, 57].

7. New therapeutic drug targets for osteoporosis

Apart from tremendous progress in the therapeutic measures currently available for the check of osteoporosis, the disease still lacks complete eradication and immediate effective relief. The side effects, e.g., in bisphosphonate-based treatment, adverse effects like femoral fractures and jaw osteonecrosis, etc. are often observed. Moreover, instances of osteosarcoma are also reported in the anabolic therapies like parathyroid infusions [56, 58]. Hence, the hunt for the novel drugs that are specific in action is still continued. In the past few years, promising research on the topics related to functional genomics and system biology has emerged as a powerful remedial tool. Within this regard, RNAi (RNA interference) can serve as a new approach of therapeutics in combating bone associated injuries. miRNA-based gene antagonism is one such influential arena in the RNAi technology. miRNAs can interact well with genes or proteins involved in the process of osteogenic differentiation and mineralization.

7.1 MicroRNAs (miRNAs)

miRNAs, a category of small non-coding RNAs, are basically 21–23 nucleotides in length. They regulate the gene expression by interacting and degrading the complementary mRNA counterparts. Additionally, they also control the expression at the protein level via the mode of translational repression of the selected proteins. For the suppression of gene, miRNAs mediate mRNA degradation, mRNA decay, insulation in P bodies and mRNA deadenylation [59]. At the protein level, miRNAs act via inhibiting the initiation or elongation steps of the translation. miRNA might also cause ribosome drops and degradation of the nascent protein chain [59]. miRNAs were discovered in the year of 1990 as regulators of gene expression for the

developmental processes in the Caenorhabditis elegans [60]. Interestingly, it has been stated that miRNA targets one-third of the genes in the human genome [61]. miRNAs are also found in extracellular fluids apart from the cells. Further, they are regarded as highly conserved elements among plant and animal kingdoms. In the context of nomenclature for the miRNAs, the preface "miR" is succeeded by a number that represents the order of naming, i.e. among miR-150 and miR-180, 150 represents the fact that it is discovered before 180 was found and named [62].

7.2 Biogenesis of miRNA

The miRNA synthesis can be briefly summarized in the following points (**Figure 4**) [63]:

a. The miRNAs genes are generally transcribed by RNA Polymerase II as pri-miRNA (primary-miRNA) in the animals.

b. A pri-miRNA may encompass one to seven miRNA precursors.

c. Enzymes Drosha and Pasha present in a microprocessor complex cleave the long pri-miRNA to shorter pre-miRNA with 2 nucleotide overhangs at 3′ end and 5′ phosphates.

d. Finally, a nucleocytoplasmic shuttle protein viz. exportin translocates the pre-miRNA to the cytoplasm.
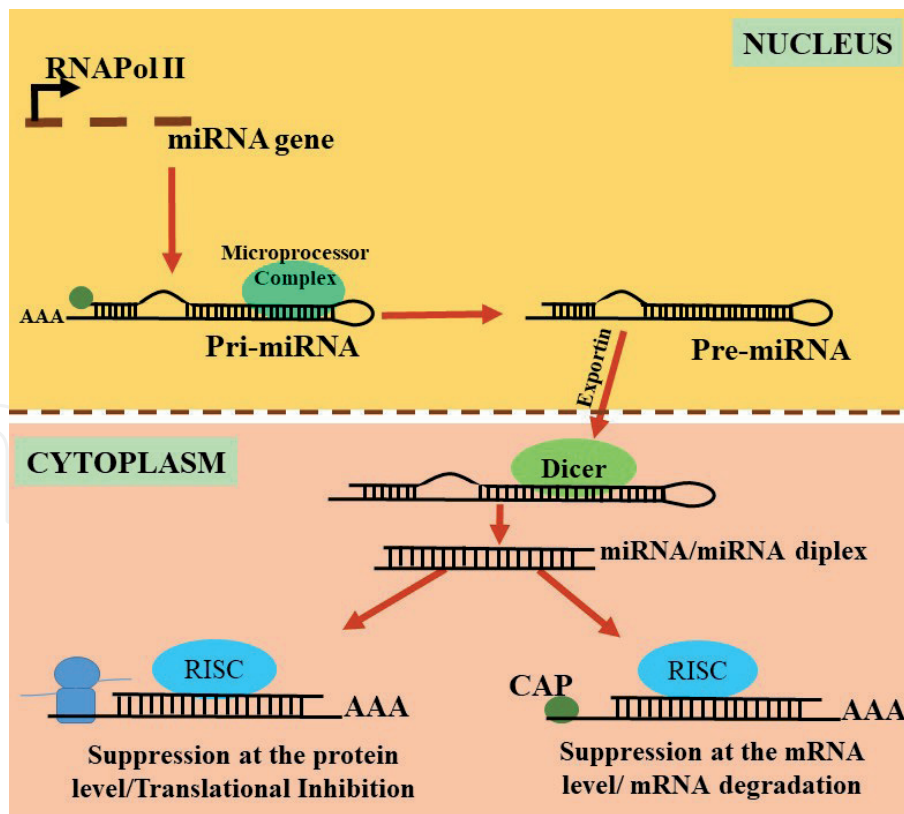
Figure 4.
Synthesis pathway for the miRNA. miRNA gene is synthesized by RNA polymerase II in the form of a primary transcript (Pri-miRNA). Pri-miRNA is acted upon by microprocessor complex (Drosha and Pasha) resulting in the formation of pre-miRNA. From the nucleus, pre-miRNA is transported to the cytoplasm by the shuttle protein exportin. In the cytoplasm, dicer targets the pre-miRNA and splices it to miRNA-miRNA duplex. The dicer cleavage is linked with the unwinding of the duplex and only one strand is selected to be incorporated into the RISC complex. In the RISC complex, miRNA executes its action either on mRNA via mRNA degradation or at the protein level by translational repression.

e. RNase III enzyme called Dicer slices the pre-miRNA into miRNA-miRNA duplex in the cytoplasm.

f. The Dicer mediated cleavage is usually associated with the unwinding of miRNA duplex wherein one miRNA strand (guide strand) is selected to be incorporated in the RISC (RNA induced silencing complex), and the other miRNA strand (passenger strand) is frequently degraded. The RISC contains Dicer, miRNA, argonaute and other accessory proteins.

g. Argonaute proteins generally interact with the mature miRNA and prepare it in the correct orientation for the subsequent binding with mRNA.

h. Perfect base pairing between the miRNA and cognate mRNA leads to degradation of the target mRNA while imperfect or partial complementarity usually results in the suppression at the protein level.

8. miRNA regulation in osteoblast proliferation

Most often, miRNA binds to 3′UTR regions of the genes and executes its action. miRNAs are generally regarded as post-transcriptional regulators that check the process of proliferation, apoptosis, differentiation and development [64]. Several miRNAs are documented to regulate the process of osteogenic proliferation, such as excessive expression of miR-221 and miR-215 in the mouse osteoblast cells encourages the proliferative capacity of the cells [65, 66]. Likewise, downregulation of miR-185 results in the declined osteoblast proliferation [67]. Besides, increased expression of miR-495 in osteoblasts results in diminished proliferation and stimulated apoptosis in the cells [68].

9. miRNA regulation in osteoblast differentiation

Differentiation of the osteoblast cells is an essential facet for the development of the adult skeleton. Most importantly, miRNAs have great potential to act against

miRNA	Target gene	Targeted signaling pathway	Reference
miR-433-3p	DKK1	Wnt/β-catenin	[69]
miR-208a-3p	ACVR1	BMP	[70]
miR-1187	BMPR2	BMP2	[71]
miR-29a	Histone deacetylase 4	β-catenin	[72]
miR-590-5p	Smad7	BMP-Smad-Runx2	[73]
miR-450b	BMP-3	BMP	[74]
miR-135	Smad5	BMP-Smad	[75]
miR-34c	Notch1	Delta-Notch	[76]
miR-224	Smad4	BMP-Smad	[77]
miR-21	HIF-1α	PTEN/PI3K/Akt	[78]

Table 2.
Role of miRNAs in the osteogenic differentiation by acting on various components in the bone signaling pathways.

or in favor of the genes that are involved in the process of bone differentiation (**Table 2**). miRNA usually targets genes that are participating in the osteo-signaling resulting in the bone differentiation.

10. miRNA regulation in bone resorption

miRNAs not only regulates the osteoblastogenesis but also sustains the bone disruptive processes by acting on the genes or proteins partaking in the signaling pathways that are functional inside the osteoclast (**Table 3**). Understanding the miRNA mediated regulation of osteoclastic differentiation will highlight the mechanism behind the differentiation process for the osteoclasts in the bone [84]. Initiating signal (binding of RANKL to the receptors) stimulates various downstream pathways (PI3K, NFK-β, MAPK) that on activation of distinct transcription factors (c-Fos, NFATc1, PU.1) control the osteoclast differentiation [84].

miRNA	Target	Effect	Reference
miR-503	RANK	Represses osteoclast formation in PBMC	[79]
miR-141	Calcr	Suppresses osteoclast differentiation, increases bone mineral density	[80]
miR-29a	RANKL and CXCL12	Decreases osteoclast formation and controls osteoporosis	[81]
miR-124	Nfatc1	Represses osteoclast differentiation	[82]
miR-155	MITF	Suppression of the osteoclastogenesis	[83]
miR-21	FasL PDCD4	Hinders the apoptosis of osteoclasts	[84]
miR-148a	MAFB	Encourages osteoclasts development	[84]
miR-125a	TRAF6	Restricts the formation of osteoclasts	[84]

PBMC, peripheral blood mononuclear cells; Calcr, calcitonin receptor; CXCL12, C-X-C motif chemokine 12; MITF, microphthalmia-associated transcription factor; PDCD4, programmed cell death protein 4; MAFB, MAF BZIP transcription factor B.

Table 3.
Representation of a few examples where miRNAs have played a vital role in the bone resorption.

11. miRNAs as therapeutics

Both the overexpression and inhibition of miRNA can be exploited for the development of potential therapeutics. miRNA sponges, Anti-miRNAs and miRNA masks are few strategies for the suppression of intracellular miRNAs. Anti-miRNAs are the miRNA inhibitors which are constructed as complementary to miRNA sequences. They prohibit the binding of miRNAs to the mRNA targets and relieve the gene suppression phenotype. Anti-miRNAs are specific in action as they are custom synthesized as entirely complementary to naturally existing miRNAs [85]. While, delivery of miRNA is achieved with the help of miRNA mimics, that imitates the sequence and action of miRNAs in the in vitro or in vivo systems. Furthermore, miRNA work as both oncogenes and tumor suppressors, thus contributes to the pathogenesis of several cancerous diseases. MiR-21 founds to be highly upregulated during breast tumors while the levels of miR-196a are significantly increased in the pancreatic cancers [86, 87]. Role of miRNAs is also evidently noticed in many other diseases viz. liver diseases, cardiac dysfunctions, renal failures, neurodegenerative diseases, etc. [88].

11.1 miRNA-based therapeutics in osteoporosis

During osteoporosis, the balance between bone formation and bone elimination is disrupted [89]. Bone dissolution dominates the bone formation and thereby results in the weakened matrix and compromised bone strength. miRNA attempts to correct the imbalance and preserves the bone homeostasis towards bone development during the process of remodeling. miRNAs suppress the genes or proteins involved in the biological signaling pathway and hence aid the pathway to proceed in accurate and normal fashion [90]. This normalization of the pathway further facilitates the optimum differentiation of the mesenchymal stem cells to the osteoblastic lineage. In the past few years, several investigations have emerged which conveys the role of miRNAs in the prognosis and treatment of osteoporosis (**Table 4**).

miRNA	Treatment: gain/ loss of function of the miRNA	Disease (osteoporotic models)	Effect	Reference
miR-148a	Loss of function using AntagomiR-148a	OVX mice	Diminished bone resorption and enhanced bone mass	[91]
miR-103a	AntagomiR-103a	Hindlimb unloaded mice	Neutralized the loss of bone, better bone mass	[92]
miR-31a-5p	AnatgomiR-31a-5p	Aged rats (Injections at the bone marrow cavity in the femur)	Reduced osteoclastogenesis and increased osteoblastogenesis	[93]
miR-1187	Anti-miR-1187	Ovariectomized BALB/c mice	Improved bone microarchitecture	[71]
miR-214	miR-214 sponges	OVX rat with femoral metaphysis critical size defect	Healing of critical size defect	[94]
miR-451a	Gain of function using miR-451a mimic	OVX mice	Improved bone strength and increased bone mineralization	[95]
miR-7b	miR-7b mimic	OVX mice	Augmented bone vascularization and bone volume	[96]
miR-199a-5p	miR-199a-5p agomiR	Sprague-Dawley rats	Better bone regeneration in the tibia-defects	[97]

Table 4.
Representation of the current studies where miRNAs are used as therapeutics in the treatment of osteoporosis.

12. miRNAs as biomarkers

Circulatory miRNAs that are available in the extracellular fluids, e.g., serum, plasma, tears, etc., are potent to be utilized as essential biomarkers in the bone associated issues. Circulatory miRNAs are generally secreted in the form of exosomes or microvesicles, and thus they are guarded against the action of nucleases. Blood plasma miRNAs have been reported as biomarkers in the diagnosis of Non-small-cell lung carcinoma stage I and II [98]. Further, miRNAs present in the human saliva have also been described as biomarkers during the menstrual cycle in women [99]. Serum biomarkers from the osteoporotic patients representing precise

pathological condition may serve as crucial diagnostic tools in the clinical practice. Studies in the past years have depicted the relevance of extracellular miRNAs in the plasma or serum samples from the osteoporotic patients relative to healthy controls. miRNAs viz. hsa-miR-122-5p and hsa-miR-4516 have been documented as putative markers in the diagnosis of osteoporosis [100]. Similarly, in another study, miR-21, miR-23a, miR-24, miR-93 and miR-100 are highly upregulated in the serum of osteoporotic patients [101]. Even in the investigation of postmenopausal osteoporosis, miR-422 has been regarded as an essential biomarker gene [102]. Based on several validated studies, it can precisely be concluded that miRNAs may act as useful potential biomarkers in the examination of distinct medical implications, including osteoporosis.

13. miRNA as potential new generation drugs

miRNAs are emerging as promising drugs in the pharmaceutical market. They are endogenous and hence associated with less harmful events for the body. Employing miRNAs as therapeutic targets have one key benefit that nucleotide content of the miRNAs can be easily modified by chemicals for the improved pharmacokinetics and pharmacodynamics of the potential miRNA-based drugs. Besides, miRNA has the capability of targeting multiple genes at a time. Moreover, nowadays chemical locked nucleic acid modifications are present for addressing the issues related to the susceptibility of miRNAs to the intracellular nucleases. Likewise, phosphorothioate alteration is another way of improving the efficacy of miRNAs in the in vivo systems [103]. Recently FDA approved drug, Onpattro against polyneuropathy marks the foundation of RNAi technology-based medicines in the commercial space. **Table 5** describes a few miRNA-based therapeutic compounds that are on the success path of drug development at the preclinical and clinical stages [104, 105].

miRNA	Drug format	Disease	Stage of clinical trial
miR-122	miR-122 Antisense inhibitor	HCV	Phase II
RG-012	Anti-miR-21	Alport syndrome	At the initiating stage of the phase II trial
miR-3	Mimic replacement	Cancers including hepatocellular	Phase I
Let-7	Mimic Replacement	Cancer	Preclinical
miR-103/105	miR-inhibitor	Insulin resistance	Preclinical
miR-10b	miR-10b inhibitor	Glioblastoma	Preclinical

Table 5.
List of few miRNAs which are presently in the development.

14. Conclusion

In healthy body conditions, miRNAs are expected to assist in the maintenance of a regulated balance between the osteoblast-based bone-forming activity and osteoclast dependent bone-resorbing activity. This balance is dependent on the action of miRNAs on the biochemical signaling pathways operating inside the bone. While, during pathologies, the aberrant expression of the miRNAs due to misregulated

bone signaling comes into existence. The upregulated or downregulated miRNAs during osteoporosis may serve as biomarkers or gene therapy targets respectively in the management of bone associated injuries including osteoporosis. As miRNAs are expressed in tissue specific manner, therefore miRNA-based biomarkers can differentiate between variable bone-related medical conditions, i.e. defects in bone fragility, reduction in bone mass density, osteoclast malfunctioning, etc. In addition, miRNA bears pleiotropic nature that favors controlling the diseases that have no efficient treatments as yet. Advances in the number of reports regarding miRNA therapeutics in osteoporosis display massive translational utility of miRNAs in the clinical practices. miRNAs are naturally occurring and expected to pose nil or few side effects to the body. The access of the first miRNA mediated therapy against hepatitis C virus infection (HCV) in the clinical phase has fulfilled the hopes for the success of miRNAs as potential therapeutic agents in the drug market. To conclude, miRNAs can undoubtedly be addressed as new generation drugs for the efficient and effective check of osteoporosis.

Conflict of interest

The authors declare no conflict of interest.

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