

MicroRNA Based Therapy and Osteoporosis: A Review of a Novel Therapeutic Agent from Diagnosis to Treatment

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Abstract

Disequilibrium between bone resorption and bone formation may cause osteoporosis that reduces bone integrity and physiological function of skeletal system. Osteoblast and osteoclast genesis are two major of biological events that act in bone turnover and dynamic rate of bone remodeling. Ample evidences have been revealed that RANK-L/OPG, Wnt and BMP Pathways are crucial pathways involved in osteoporosis. Treatment of osteoporosis is becoming important task in post menopause women and old people. Current treatment strategies with osteoporosis drugs are mainly by inhibiting the bone-resorbition. However, these synthetic medicines have limitless side effects. Several studies have established the important of a group of small non-coding RNAs (MiRNAs) which involve in pathogenesis osteoporosis, bone remodeling, osteoblast differentiation and osteoclast formation and has consider as a gold biomarker for osteoporosis treatment. The pathogenicity factors of osteoporosis, pathways involved in the disease and potential replacement treatment have been emphasized in this paper.

Keywords: Osteoporosis therapeutic strategy; Wnt signaling pathway; RANKL/OPG signaling pathway; BMP pathway; MiRNAs

Introduction

Osteoporosis can be defined as a disease of bone metabolism associated with low bone mass and micro-architectural deterioration, which increase the risk of bone fracture [1]. Etiologically this disease has a complex interaction between genetics and environmental factors and lifestyle with an disequilibrium between two critical biological processes namely osteoblastic bone formation and osteoclastic bone resorption [2,3]. Approximately 28 million individuals are involved and estimated 1.5 million fractures take place annually in United States [4]. Disease is categorized as primary, which is closely correlated with age and sex, and secondary, that occurs at any age which effecting men and women equally. At the molecular level, three critical pathways including Wnt Pathway, BMP Pathway and RANK-L/OPG Pathway are involved in osteoporosis and bone remodeling process. Osteoporosis basing to be a serious public health issue especially for these two groups of population, rarely elderly and post-menopausal osteoporosis women [4]. Although drug selection for osteoporosis is limited but evidence has demonstrated the crucial role of MiRNAs in osteoporosis treatment [5]. This review highlighted the pathogenesis of osteoporosis, molecular mechanisms involved in the disease and comprehensive review of osteoporosis treatment using chemical drugs and MiRNAs therapy as a potential novel therapeutic agent in osteoporosis.

Literature Review

Pathogenesis of osteoporosis

Osteoblastogenesis and impact osteoclastogenesis are two crucial biological events of bone turnover which can reflect the dynamic rate of bone remodeling [6,7]. Cumulating evidence suggested that three major molecular pathways including RANK-L/OPG Pathway, Wnt Pathway and BMP Pathway are involved in pathogenesis of osteoporosis.

Receptor activator of NF- κ B ligand- osteoprotegerin (RANKL-OPG Pathway)

Various factors, receptor activator of NF- κ B ligand (RANKL) and macrophage colony stimulating factor (M-CSF), are secreted from osteoblasts. These factors perform like a regulator in the process of

differentiation of osteoclast progenitors to mature osteoclast. The cells are detected by expression of various markers such as calcitonin receptor [8-11], and alpha v beta 3 integrin chain (α v β 3) [12]. The process has activates various signaling pathways such as NF- κ B with the involvement of RANK and RANKL. Many signaling pathways could be activated during the regulation of osteoclastogenesis, such as NF- κ B, via binding of RANK and RANKL, important regulator of osteoclast precursors along with CD11b, CD14 and cFms [13,14]. Inhibition of osteoclastogenesis occurs when Osteoprotegerin (OPG), a decoy protein secreted by osteoblasts, could bind to RANKL and RANK. Involvement of RANKL-OPG play a crucial role in the cross-talk between osteoblast-mediated bone formation and osteoclast-mediated bone resorption [13,14].

Wnt signaling pathway

Wnt pathway consists of several signaling proteins that have critical role in multiple biological processes such as cell survival, migration, apoptosis, cell proliferation and differentiation [15] that is involved in bone homeostasis, bone remodeling process and proliferation and differentiation of osteoblast progenitors [16-18]. Osteoblasts formation and bone-resorbing osteoclast are terminally differentiated cells with short lives. Therefore, replacement of both with a new ones from mesenchymal and hematopoietic stem cells are necessary [19,20]. Wnt/ β -catenin also known as canonical wnt signaling pathway. It generates osteoblasts through the promotion of differentiation to osteoblast lineage from pluripotential mesenchymal stem cell [21]. In response to the pathway, cell expressed with osterix-1 (OSX1)

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develops to osteoblast. The life span of mature osteoclasts increases by prevention of the apoptosis process in both β -catenin dependent and independent pathways [22]. On the other hand, wnt/ β -catenin signaling able to decrease differentiation of osteoblast through encouragement of production and secretion of osteoprotegerin (OPG). OPG is a natural antagonist of RANKL [23]. It should be noticed that RANKL which is produced by primarily osteocytes is essential for the differentiation, survival and function of osteoblast and bone resorption [24]. In osteoclast production, bone marrow macrophages (BMMs) are differentiating into positive pre-osteoclasts and tartrate-resistant acid phosphatase (TRAP). These compounds are crucial in forming the mature osteoclast [25]. Wnt signaling has effect on the inhibiting of osteoclast activity by extracting Frizzled-related protein 1 (Sfrp1). As Sfrp1 is a binding site of Frizzled protein, it binds to RANKL competitively. This complex down-regulates the bone resorption [26,27] and decrease the level of β -catenin in osteoblast progenitors, hence caused the bone loss [28].

BMP pathways

A group of cytokines known as Bone morphogenetic proteins (BMPs), belong to transforming growth factor- β (TGF- β) superfamily. BMP is managed by RSmads and Co-Smad. The interaction of BMPs and responsive receptors influences the phosphorylation of Smads, thus enhance osteogenesis [29,30]. During this process, Runx2, a transcription factor connects many signal transductions in bone remodeling and osteoclast differentiation activated and regulates the genes associated to bone formation. It is necessary for activation of Smads by BMPs as well [31]. Ample evidence revealed that Runx2 enhances the level of PI3K/Akt [32]. During osteoblast differentiation, another mediator molecule known as osterix. This molecule act as a mediator for Runx2 that play an important role in managing bone formation and bone resorption [33]. Several successful project implementations involving of HDAC family members in bone remodeling including HDAC1 and HDAC3 (mostly found in bone tissues) [34,35].

Current treatment of osteoporosis

Currently, there is a limitation of drug selection for osteoporosis treatment. Those problems are due to various potential and toxicity issues. Thus, establishing a new approach for osteoporosis has become a great interest. A current treatment, Calcitonin, was related to cancer for long-term users, thus was eliminated as one of the treatment cycle in the Europe countries [36]. It leave Teriparatide (PTH 1-34) and PTH 1-84 (stimulate the formation of new bone), and Biphosphonates (anti-resorption) as the remaining choices [37].

Anabolic agents in osteoporosis treatment

Anabolic agents are a class of osteoporosis treatment drug with many kinds of therapeutic targets that increase bone mass by directly stimulating new bone formation [38-48].

Parathyroid hormone (PTH) analogs: Human recombinant PTH is the only anabolic therapy that permitted to treat osteoporosis, such as PTH1-34 and PTH1-48 [49]. The most common side effects of PTH analogs are mild asymptomatic hypercalcemia and hypercalciuria whereas PTH-related protein cause hypercalcemia in malignant patients [49]. The main limitation for these kinds of drugs is the economic factor because PTH analogs are expensive to produce.

Potential therapeutic targets of osteoporosis: Having knowledge of the micro environmental control process that able to regulate bone modeling and bone remodeling is necessary in planning therapeutic for prevention and treatment of bone fragility. Resorption inhibitors

are new class of osteoporosis treatment agents that are being developed based on their action mechanisms. ODN, DPH, GSK-3 inhibitor and DDK-1 inhibitor are examples of this new class of drugs for osteoporosis treatment [49].

Cathepsin K inhibitors

Cathepsin K inhibitors, such as Odanacatib (ODN), are anti-resorptive agents [38,39] that digest the type I collagen in resorption pits. They targeting the selective osteoclast digestive enzymes making their anti-resorptive effect are tolerable compare to more potent anti-resorptive agents [40-42]. ODN has intermediate effect observed on continuous bone resorption and transient down regulate in bone formation [40-42]. Another experiment was done by a group of researchers that revealed cathepsin K deficiency led to maintenance of, or an increase, in bone formation in mice, rabbits and monkeys [43,44]. Odanacatib is undergoing phase III clinical trials in postmenopausal women and older men. Another example, ONO-5334, acts in suppressing the bone resorption is experiencing Phase I and II clinical trials [45]. Although, such these inhibitors have a successful clinical trials but most of them express multiple kinds of side effects [45].

Inhibition of DDK-1(Dikkoff-1)

The inhibition of canonical Wnt signaling pathway take places by producing a trinary complex between LRP5, DKK-1 and DKK receptor. This kind of interaction encourages the fast internalization and reduction of LPR5 (low density lipoprotein receptor-related protein 5). Later, it causes the inhibition of Wnt [46]. Contradiction to that, inhibition of the DKK-1 and LRP5 activates the Wnt signaling. In a study have been shown that using of anti-DKK-1 antibodies trigger increase number of osteoblasts and down regulate the number of osteoclasts [47]. This study was performed on a mouse model subjected with multiple myeloma.

Wnt/ β catenin signaling pathway activators and inhibitors in osteoporosis

Binding of Wnt to the receptor and LPR5/6 in osteoclasts prevent the formation of GSK-3 (intracellular glycogen synthase kinase-3) in Wnt signaling pathway [22]. This inhibition of the signaling increases the level GSK3 that can inhibit the breakdown of catenin. This mechanism causes transcriptional co-activation of genes integral to bone formation [22]. Most of the bone active drugs mainly target at Wnt signaling pathway, which includes Wnt antagonist inhibition like sclerostin, DDK1 and SFRP1, along with neutralization of antibodies and suppression of glycogen synthase kinase 3 β (GSK3 β) which promotes phosphorylation and degradation of B-actin [48]. Number of endogenous antagonists constrains the Wnt/ β -catenin pathway, namely Wnt-inhibitory factor and members of the secreted frizzled-related protein family. These endogenous antagonists inhibit the Wnt/ β -catenin pathway and later cause the down-regulation of bone formation. Same reaction occurs for LRP5/6 inhibitors that block this signalling [48].

Osteoporosis linked MicroRNAs (MiRNAs)

MiRNAs able to control gene expression at the post-transcriptional level by targeting the 3' un-translated region of mRNA [50]. They have ability to regulate various biological pathway including cells development, hematopoiesis, pathophysiological process, organogenesis, apoptosis, cell differentiation and tumor genesis [51]. Various studies indicated that MiRNAs regulate osteoclastogenesis and are involved during the pathogenesis of osteoporosis as well [52]. Most of the MiRNAs control the proliferation and differentiation of osteoblast. Some of MiRNAs play a crucial role in controlling the differentiation of osteoclast [53]. On the basis of previous investigation, aberrant MiRNAs expression

revealed a close association with osteoporosis, for example, miR-2861 as a novel MiRNAs able to target histone deacetylase 5(HDAC5), a negative regulator of RUNX2, can cause familial osteoporosis [54]. Polymorphisms in miR-146a and miR-14b target sites (able to target FGF2) are significantly associated with femoral neck bone mineral density [55]. Table 1 shows a brief history of MiRNAs functions and their targets in osteoporosis [54-86].

Discussion and Conclusion

In recent years, osteoporosis becoming a serious public health problem in post-menopausal osteoporosis women and old population. Despite of an increasing number of advanced researches performed to gain better understanding of the pathogenesis and molecular pathways involved in osteoporosis grey zone is still exist in the knowledge of osteoporosis association with variety of molecular mechanisms. Recent approaches in managing osteoporosis are mainly focused on the bone-

MicroRNAs	Target gene(s)	MicroRNA Function	References
miR-2861	HDAC5	Promotes osteoclast differentiation and primary osteoporosis induction	[54,56]
miR-3960	HOXA2	A repressor of RUNX2 and acts as a regulatory role in osteoblast differentiation	[57]
miR-223	Ago2, DGGR8, NFI-A, RANKL, TNF- α	Inhibit of osteoclast marker and transcription factor expression, decrease osteoclastogenesis (decrease bone resorption)-considered as a therapeutic target for a range of bone metabolic disorders	[58]
miR-206	Cx43 (Connexin43)	Induction of osteoblast differentiation	[59]
miR-29 miR-27 mir-335-5P	Wnt –Signaling pathway (DDK1, Kremen2, SFRP)	Up regulated , regulate osteoblast differentiation by suppresses of key wnt signaling antagonists	[60,61]
miR-21	PDCD4, c-Fos	Down regulation of programmed cell death-4	[58,62]
miR-93 miR-96	SP7 transcription factor 7 Zfp521	Regulatory role in stem cell and bone marrow mesenchymal stem cell differentiation	[63,64]
miR-199a miR-26a	SMAD1	Regulatory role in skeletogenesis and its pathogenesis	[65]
miR-126	VCAM1, HOXA9,	Regulatory role in skeletogenesis and its pathogenesis	[66]
miR-140	HDAC4	Regulatory role in skeletogenesis and its pathogenesis	[67]
miR-125b	ERBB2	Regulatory role in skeletogenesis and its pathogenesis, considered as a potential non- invasive biomarker for postmenopausal osteoporosis	[67]
miR-29b miR-133a, mir-135b	HDAC4, TGF β 3, ACVR2A, CTNBNB1PI, DUSP RUNX2, SMAD5	Skeletogenesis development	[68]
miR-638 miR-663	JUN, FOSB, SP3, MYC	Regulate osteoblastogenesis	[69]
miR-29b	MMP2, c-Fos, HDAC4, TGF β 3, ACVR2A, CTNBNB1P1and DUSP	Down regulated in RNKL induced osteoclastogenesis that inhibits osteoclast differentiation	[70]
miR-378 miR-146a	NFIA	Down regulated in osteoclast differentiation that able to inhibits differentiation	[71]
miR-133a	CXCL11, CXCR3, SLC39A1	Up-Regulated in post-menopausal osteoporosis, Its overexpression (targeting SLC39A1) is negatively correlated to osteogenic differentiation of hMSCs	[72]
miR-338-3P	RANKL	Induction of osteoblast	[73]
MiR-21	RANKL	Induce osteoclastogenesis	[74]
miR-503	RANKL	Inhibits RANKL-induced osteoclast differentiation in post-menopausal osteoporosis women	[75]
miR-150-3P	β -catenin	Suppressing of osteogenic differentiation through downregulation of β -catenin	[76]
miR-125a	TRAF6	Down regulated and Inhibits osteoclastogenesis and involve in metabolic disorders	[77,78]
miR-221	RUNX2	Osteoclast differentiation	[79]
miR-125b miR-30	ERBB2 SMAD1 and RUNX2	Unregulated in postmenopausal osteoporosis women, as a potential biomarker Unregulated in postmenopausal osteoporosis women	[80]
miR-34C	LGR4	Promote osteoclast differentiation	[81]
miR-705 miR-3077-5P	HOXA10 RUNX2	Inhibit mesenchymal stem cells (MSCs) osteoblast differentiation and promote adipocyte differentiation	[82]
MiR-133b	RUNX2, ALP, OCN (osteocalcin)	Negatively regulates the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cell	[83]
miR-148a miR-422a	MAFB TOB2, PAG1, IGF1, CD226	Unregulated during osteoclast differentiation and promote osteoclastogenesis	[78,84]
miR-146a, miR-146b	FGF2	Femoral neck bone mineral density, aberrant miR Expression	[80-82]
miR-9	AMPK Signaling pathway	Regulation of osteoblast differentiation and angiogenesis	[83]
miR-7b	DC-STAMP	Osteoclast differentiation by suppressing NFATC1 and C-Fos signaling	[84]
miR-106b	RANKL	Inhibits osteoclastogenesis and osteolysis	[85,86]

Table 1: MiRNAs involved in osteoporosis pathogenicity, osteoblast formation and osteoclast differentiation.

resorbing drugs accompanied with limitless adverse effects. Although, osteoporosis treatment based MiRNAs is becoming excellent options, but further investigation into MiRNAs role especially in controlling the differentiation of osteoclast need to be considered.

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