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Review article

Regulation of Runx2 by MicroRNAs in osteoblast differentiation

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ABSTRACT

Bone is one of the most dynamic organs in the body that continuously undergoes remodeling through bone formation and resorption. A cascade of molecules and pathways results in the osteoblast differentiation that is attributed to osteogenesis, or bone formation. The process of osteogenesis is achieved through participation of the Wnt pathway, FGFs, BMPs/TGF-β, and transcription factors such as Runx2 and Osx. The activity and function of the master transcription factor, Runx2, is of utmost significance as it can induce the function of osteoblast differentiation markers. A number of microRNAs [miRNAs] have been recently identified in the regulation of Runx2 expression/activity, thus affecting the process of osteogenesis. miRNAs that target Runx2 corepressors favor osteogenesis, while miRNAs that target Runx2 coactivators inhibit osteogenesis. In this review, we focus on the regulation of Runx2 by miRNAs in osteoblast differentiation and their potential for treating bone and bone-related diseases.

1. Introduction

Bone is a strong, mineralized, connective tissue that aids in motion, protects vital internal organs, and stores essential ions like calcium and phosphate [1]. Long bones house the most important reservoir of stem cells, i.e. bone marrow, which is responsible for the production of hematopoietic tissues [2]. Initiated by mesenchymal condensation, bone formation occurs by the inclination of cells towards osteochondroprogenitor lineage [3]. Bone cytology includes osteoblasts, the bone-forming cells; osteoclasts, the 'scavengers' of the bone; osteocytes, the pillars of the bone structure; and bone lining cells, the reservoir of determined osteogenic cells [4,5]. In addition to bone cells, the bone consists of a unique and complex organic structure, called the extracellular mineralized matrix, which behaves as a scaffold for mineralization and provides shape, support and rigidity to the bone [6]. The organic component of bone comprises collagenous proteins; type I collagen (Col−I) is more abundant than collagen types III, V, and IX. The less abundant non-collagenous proteins contain proteoglycans, glycoproteins, osteonectin, thrombospondin, and fibronectin. The inorganic component of bone is made up of calcium hydroxyapatite and phosphate [7–9].

Following the mesenchymal stem cell (MSC) or progenitor proliferation phase, a significant expression of Runx2, Col-I and alkaline phosphatase (ALP) occurs. Increased expression of Osterix (Osx) and secretion of bone matrix proteins such as osteocalcin (OCN), bone sialoprotein (BSP) I/II and Col−I, are also needed to facilitate the morphological changes and transformation of preosteoblasts into mature osteoblasts [10–14]. Different signaling pathways and factors are adapted at different stages of osteoblast differentiation. For instance, during endochondral bone formation, Indian hedgehog (Ihh) stimulates differentiation of mesenchymal progenitor cells [3]. Notch signaling promotes differentiation of preosteoblasts into osteoblasts, but suppresses preosteoblast formation from MSCs. For membranous as well as endochondral bone formation, Wnt canonical (β-catenin-dependent) and noncanonical (β-catenin-independent) pathways are crucial [13]. Two vital transcription factors, Runx2 and Osx, orchestrate MSCs' influence on the formation of osteoblasts, osteocytes, osteoclasts and bone lining cells [14]. Runx2, the Runt-related transcription factor, is the master transcription factor that communicates with the promoters of its target genes, facilitated by its Runt domain [10,15]. Runx2 has the capacity to upregulate Col−I, ALP and OCN genes [16]. Positive as well as negative regulation of Runx2 is pivotal in the phenomena of bone formation [17,18]. Runx1 and Runx3, the other members of the Runt family, are also involved in the stimulation of osteogenic genes [19].

Positive regulators of Runx2 include: bone morphogenetic proteins (BMPs); fibroblast growth factors (FGFs); and histone acetyltransferases (HATs) such as p300, CBP (CREB binding protein), MORF, MOZ (monocytic leukemia zinc finger protein), and PCAF (p300/CBP-

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associated factor) [20]. Repressors of Runx2 include Snail1 protein, twist transcription factor, TLE (transducin-like enhancer), mSin3A, and histone deacetylases (HDAC-3, -4, -5, and -6) [21,22]. Runx2 expression decreases during the maturation of osteoblasts and its regulation by different factors is imperative to osteoblast lineage differentiation [22]. With the emergence of epigenesis and associated regulatory mechanisms, miRNAs are now considered one of the most potent regulators at the post-transcriptional level of gene expression.

miRNAs are non-coding RNAs that are 20–22 nucleotides long and regulate gene expression at the post-transcriptional level by hybridizing with mRNA at the 3′UTR (untranslated region), thereby silencing the genes and consequently governing protein synthesis [23–25]. Complementarity of miRNA to mRNA controls the regulation of the gene by either blocking the translation or cleaving the mRNA, which is facilitated by the binding of the 5′ end of the miRNA to the 3′ UTR of the mRNA [26,27]. miRNA genes loci are predominantly identified in the intronic regions of non-coding and coding genes, but some also originate in the exonic regions of structural genes [28]. Mature miRNA is derived from a primary transcript (~80 nt) processed by an RNase III enzyme, Drosha; followed by the cleavage of a hairpin loop to produce a precursor transcript by another RNase III enzyme, Dicer, in the cytoplasm. One strand from the miRNA duplex later gets incorporated into the RNA-induced silencing complex (RISC) to target and regulate the appropriate mRNA [28–30]. miRNAs are responsible for controlling various cellular processes like cell proliferation, differentiation, developmental processes, and survival [31]. In osteoblasts, miRNAs play a pivotal role in post-transcriptional regulation of genes that participate in differentiation. For example, miR-204 and miR-133 behave as negative regulators of osteogenesis by directly targeting Runx2 [16]. miR-433 inhibited osteoblast differentiation in BMP-2-induced mouse MSCs (mMSCs, C3H10T1/2) by targeting Runx2 [32]. miR-2861 and miR-3960 have been shown to promote Runx2 expression by inhibiting HDAC-5 and HoxA2 (Homeobox A2), respectively, in mMSCs (ST2) [33]. In this review, we focus on a number of miRNAs that have been identified to regulate osteoblast differentiation via Runx2 directly or indirectly, and the potential of using these miRNAs for treating bone and bone-related diseases.

2. Runx2

Runx2 is known by various names such as Cbfα1 (core binding factor alpha 1), OSF-2 (osteoblast specific factor-2), PEBP2α (polyoma enhancer binding protein 2α), and AML-3 (acute myeloid leukemia-3); it is essential for the expression of osteogenic differentiation genes [34–36]. Runx2 is one of three members of the Runx family that have the common evolution-conserved Runt domain and facilitate communication with other proteins [37–40]. All three members (Runx1, Runx2, and Runx3) play pivotal roles in skeletal development [40–42].

The function of Runx2 is attributed to the structure of the protein encoded by its mRNA sequence (Fig. 1). Runx2's various domains/sites facilitate bone remodeling as each distinctive function is based on its interaction with other proteins and DNA sequences. Positive and negative factors interact with Runx2 to elicit a synergistic functional effect that leads to many developmental changes in the cell [40,43]. The Runx2 gene is 227,766 nucleotides long, which constitutes 8 exons that are vital for translation. Two types of isoforms are produced based on the transcriptional function of the two promoters of the gene, leading to either MASNS or MRIPV pentapeptides. The former, encoded by all 8 exons, is produced from the distal promoter (P1), and the latter, encoded by exons 2–8, is formed by the proximal promoter (P2). Several domains of translational significance are coded by these exons; the Runt domain is encoded by exons 2, 3 and 4; the nuclear localization signal (NLS) is encoded by exon 5; the nuclear matrix targeting signal (NMTS) and VWRPY (TLE/Groucho interactions) are encoded by exon 8 (Fig. 1).

3. miRNAs in Runx2-mediated osteoblast differentiation

3.1. Direct regulation of Runx2 by miRNAs

miRNAs are endogenous single-stranded RNA molecules that complement a target mRNA of a protein-coding gene. Runx2 is targeted by an array of miRNAs that directly terminate its expression; facilitated by direct binding and interaction of the miRNA with the Runx2 mRNA in the cytoplasm. Upon BMP-2 induction, miR-433 levels are reduced, but overexpression of miR-433 decreases Runx2 levels, evincing the direct relationship between miR-433 and Runx2 [45]. miR-193b and miR-455 directly target Runx2 and regulate chondrogenesis [46,47]. miR-203 downregulates Runx2, MMP-13 (matrix metalloproteinase 13), IL-11 (interleukin 11), and PTHrP (parathyroid hormone-related protein) expression, through direct target inhibition [48]. Overexpression of miR-103a decreases the concentration of Runx2 protein in bone-related disorders [49]. Osteogenic lineage commitment is controlled by expression of a set of 11 miRNAs (miR-23a, miR-30c, miR-34c, miR-133a, miR-135a, miR-137, miR-205, miR-217, miR-628-3p, miR-335, and miR-338) that potently target Runx2 and downregulate osteogenesis in C3H10T1/2, C2C12, NIH3T3, and 3T3-L1 cells [50–54]. In another study, it was shown that miR-155 downregulates Runx2 protein expression rather than altering only the mRNA expression in MEF and C2C12 cells [55]. miR-204 and miR-211 significantly reduce the expression of Runx2 protein, which affects osteoblast differentiation [56]. In the same manner, miR-590 directly targets Runx2 mRNA and inhibits its expression [57]. It was observed in a recent study that treating human osteoblastic cells (MG-63) with parathyroid hormone (PTH), increased expression of miR-6797 and a long non-coding RNA (SUPT3H-1:16), that in turn protected Runx2 from the respective miRNAs' direct target inhibition through a sponging mechanism [58]. Table 1 provides information on direct targeting of Runx2 by miRNAs that affects osteoblast differentiation.

3.2. Indirect regulation of Runx2 by miRNAs

3.2.1. Coactivator-dependent repression of Runx2 by miRNAs

The process of osteogenesis is dictated by several extracellular

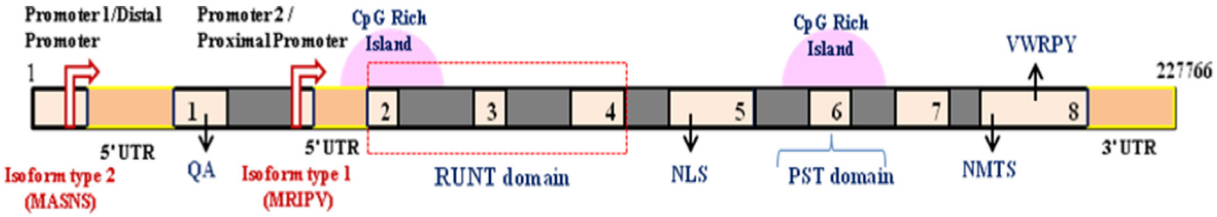

Fig. 1. Genomic organization of Runx2. The Runx2 sequence is 227,766 nucleotides long, constituting eight exons (numbered 1–8). Several domains of translational significance are coded by these exons, namely RUNT (exons 2–4), NLS (exon 5), NMTS (exon 8), VWRPY (exon 8) and CpG rich islands are encoded by exons 2 and 6. Before the RUNT domain the QA domain is present and in between the NLS and NMTS, the PST domain is present. Two types of isoforms MASNS or MRIPV are produced based on the transcriptional function of either P1/Distal promoter (exons 1–8) or P2/Proximal promoter (exons 2–8), respectively [22,42,44].

Table 1
A functional role of miRNAs that directly target Runx2.

miRNAs	Functional characteristics	Model	References
miR-204, miR-211–204 miR-211	Negatively regulates Runx2 resulting inhibition of osteogenesis and promotion of adipogenesis	Human mesenchymal stem cells (hMSCs), Mouse bone marrow mesenchymal stromal cells (BMSCs)	[56]
miR-23a, miR-30c, miR-34c, miR205, miR-217	Downregulates Runx2 that leads to inhibition of osteogenic differentiation	Mouse mesenchymal stem cells (C3H10T1/2), Mouse myoblasts (C2C12)	[51]
miR-433	Decreases Runx2 expression via direct inhibition upon BMP-2 treatment inducing osteoblast differentiation	C3H10T1/2	[32]
miR-133a	Involves in osteogenic differentiation of vascular smooth muscle cells mediated through β-glycerophosphate	Primary mouse vascular smooth muscle cells (VSMCs)	[178]
miR-135, miR-203	Downregulates Runx2 expression and its target genes such as MMP13, IL-11, PTHrP thus controlling in bone metastasis	Human breast cancer cells (MDA-MB231)	[48]
miR-455-3p	Involves in the regulation of early differentiation of chondrocytes by downregulating Runx2	Mouse teratocarcinoma cells (ATDC5)	[47]
miR-155	Regulates osteogenesis through induction of BMP pathway by BMP9	C2C12, Human embryonic kidney 293 cells (HEK-293)	[55]
miR-590-3p	Directly targets Runx2 and promotes apoptosis in breast cancer cells thus controlling in bone metastasis	MDA-MB231	[57]
miR-6797-5p	Acts as a negative regulator of Runx2 upon PTH-treatment	Human osteosarcoma cells (MG63), Human bone marrow stromal cells (hBMSCs)	[58]

ligands such as bone morphogenic proteins (BMPs), WNTs, and fibroblast growth factors (FGFs), that regulate the activities of various homeodomain proteins and vital transcription factors like Runx2 and Osx [11,59–64]. Additionally, Runx2 is regulated by helix-loop-helix factors, Ets factors, Zn finger proteins, and homeodomain proteins including cofactors [65–71]. While the above-mentioned regulatory mechanisms of Runx2 play a role during its gene expression, miRNAs are key players of its post-transcriptional regulation [45,56]. As mentioned earlier, the level of Runx2 protein is highly maintained during the early stages of osteoblast differentiation, until the mineralization stage when it decreases [18,43,50,72]. Therefore, there are also modulations witnessed in the expression of miRNAs that target Runx2 during different stages of osteogenesis [50]. miRNAs that target Runx2 or its activators lead to inhibition of osteogenesis, which is achieved by affecting either mRNA stability or protein expression of Runx2 [73].

BMP-7 is a positive regulator of osteogenesis and is targeted by miR-542-3p. So far, this miRNA has been used extensively as a tumor suppressor; it has been now established as a potential therapeutic agent in bone remodeling because its suppression can cause upregulation in BMP-7-induced osteogenesis [74]. miRNAs such as miR-654-5p and miR-98 inhibit osteogenic differentiation by directly targeting BMP-2 [75,76], while other miRNAs target the downstream molecules of the BMP pathway. miR-31, miR-106a, miR-148a and miR-424 inhibit Runx1/Cbfb and BMPs, consequently leading to inhibition of osteogenesis [43,77,78].

Smad1/5/8 is activated by extracellular BMPs, leading to Smad complexes formed with Smad4 that induce the expression of Runx2 and other osteogenic genes [79,80]. Multiple studies have proven the inhibitory effect of many miRNAs on BMPs and their cascade molecules. miR-222-3p, miR-155, miR-93-5p, miR-106b-5p, miR-17-5p and miR-135 interrupt the BMP signaling pathway by specifically hampering the translation of Smad5, resulting in the repression of osteogenesis [45,81–84]. The function of Smad1 is blocked by the direct binding of miR-26a, impeding Runx2 expression, and ultimately osteogenic differentiation [85]. miR-708 suppresses osteogenic differentiation of MSCs via the Smad3 protein that mediates the TGF-β signaling mechanism during the early stages of osteogenesis [86,87].

Another BMP-induced transcriptional regulation is through the Dlx homeodomain protein family, which functions either by activating expression of the Runx2 gene or other osteogenic genes independent of Runx2 [88–92]. miR-141 and miR-200a repress the translation of BMP-2-induced Dlx5, which plays a role in inducing the expression of Runx2 in mature osteoblasts [89,93]. BMP receptors (BMPR1 and BMPR2) are prerequisites for the successful signaling of BMPs and their downstream molecules [94–96]. Few studies conducted in the past showed that miR-125b targets BMPR1b in its 3′-UTR, consequently curbing osteogenic differentiation [97,98]. BMPR2 is a target of miR-153 and miR-100, which results in its downregulation [99,100]. This causes repression of osteogenic differentiation, as BMPR2 is a crucial player in the expression of Runx2. ACVR1 (Activin A receptor type 1), a pivotal protein of the TGF-β/BMP signaling pathway, effectively influences osteogenic differentiation. miR-148a and miR-208-3p target and thereby repress ACVR1, which interferes with the BMP-2-induced Smad1/5 pathway [101–103].

HOXA10 is an important determinant in the differentiation of osteoblasts from MSCs [104,105]. HOXA10 can function via Runx2, either independently or through direct targeting, leading to the promotion of other osteoblast genes like ALP, OCN, and BSP [106]. miR-705, miR-3077-5p, and miR-320 carry out the inhibitory regulation of osteogenesis through HOXA10 [107,108]. miR-214 has been established as a suppressor of osteogenic differentiation. It functions by hindering the phosphorylation of FGFR1 (fibroblast growth factor receptor 1), which in turn alleviates the phosphorylation of its downstream proteins ERK1/2 and Runx2. This FGFR-signaling pathway is of great significance in bone formation [109–112]. The FGFR2 receptor promotes osteogenic differentiation at various stages of bone development, starting from MSCs until the osteoblast stage, via ERK1/2 and PKCα signaling. miR-223 controls osteogenic and adipocyte differentiation via two mechanisms, one of which is through FGFR2; it binds directly to the 3′-UTR of the receptor and interrupts the Runx2 expression [113–115]. The other mechanism involves the attenuation of 3′ UTR of Wwtr1, a transcriptional co-activator that binds to the regulatory regions of PPARγ and Runx2, consequently leading to inhibition of osteogenesis [99,100,115,116].

miR-383 [117], miR-34s [118] miR-33a-5p [119], miR-205 [120], and miR-23a-27a-24-2 cluster [66] are among the negatively regulating miRNAs of osteogenesis that attenuate Satb2 (special AT-rich sequence–binding proteins). These miRNAs exhibit various mechanisms to suppress Satb2 viz. miR-383, miR-33a-5p and each member of the miR-23a-27a-24-2 cluster directly targets the 3′UTR of its mRNA [66,117,119]; while miR-205 partly inhibits Satb2 expression by interfering with the phosphorylation of ERK and p38 MAPK pathways [120]. Regardless, the expression of Satb2 is inhibited, and this prevents the formation of the coregulatory complex of Satb2 and Runx2 for the successful differentiation of osteoblasts [121].

3.2.2. *Corepressor-dependent activation of Runx2 by miRNAs*

Many recent advances have shown that miRNAs not only inhibit osteoblast differentiation by targeting the coactivators, but also activate osteoblast differentiation by targeting the corepressors. There is an

array of corepressors and an even bigger list of miRNAs that target corepressors. Corepressor proteins like TLE (transducin-like enhancer protein), mSin3a, and YAP (yes-associated protein) have been shown to truncate Runx2 activity by preventing it from binding to the enhancer regions of target DNA. Conserved domains WRPW and WRPY, on TLE (transducin-like enhancer protein) and Runx2 proteins, respectively, interact to repress Runx2 expression during osteoblast differentiation [122].

HDACs (histone deacetylases) are specific proteins that orchestrate the negative regulation of Runx2 [36,123]. HDAC-3 interacts with the amino terminal of Runx2 and downregulates its expression, resulting in the inhibition of bone formation [44,124]. HDAC-1 mediates downregulation of OPN through the Runx2-dependent pathway [125]. Direct target inhibition is carried out by miR-499a over HDAC-1 [126]. Looking at the prevalence and potent effect of many miRNAs like miR-224 and miR-34a in hepatocellular carcinoma and breast cancer, respectively, miRNAs could potentially enhance Runx2 expression [35,127]. During hypertrophy of the growth plate, the expression of miR-1 is significantly high, subsequently leading to direct inhibition of HDAC-4 [128]. Upon TGF-β and BMP-2 treatment, HDAC-4 is identified as a corepressor of Runx2, which is countered using miR-29a/b, miR345, miR-140, and miR-365 to reactivate Runx2 expression [128,129]. HDAC-5 behaves similar to HDAC-4. miR-2861 and miR-3960, induced by the BMP-2 signaling pathway, target HDAC-5 and Hoxa2, a homeobox protein that directly inhibits the Runx2 gene. A recent study showed that the enhancement of osteoblast differentiation could be achieved by the direct inhibition of HDAC-2 using miR-233 in MC3T3-E1 cells [130]. Another study showed that HDAC-6 interacts with the carboxy-terminal of Runx2 and mediates its repression. miR-22 targets HDAC-6, whereas miR-675 targets both HDAC-4 and -5 [131]. Runx2 is a direct target of HDAC-7 by its association with various carboxy-terminal domains on the protein [132]. To our knowledge, no specific miRNAs target HDAC-7 in bones. As already mentioned, miR-455 and exosomal miR-95 selectively decrease the amount of HDAC-8 mRNA to be translated, promoting more H3 acetylation and enhancing Runx2 expression. Though this mechanism was mainly observed in chondrogenesis, it could be investigated in osteoblast differentiation as well [133]. Even though most HDACs interact with Runx2, HDACs-9, -10, and -11 do not play a significant role in modulating Runx2 expression [132].

E3 ubiquitin ligase is an enzyme that plays an important role in directing proteasomal-dependent protein degradation by transferring a ubiquitin molecule from a ubiquitin-conjugating enzyme, E2 enzyme, to a lysine residue on the protein [134]. The downstream regulators of the BMP pathway, Smads 1, 5, 6 and 7, are considered major inducers of E3 ubiquitin ligase-dependent protein degradation by the incorporation of Smurf-1 protein (Smad ubiquitin regulatory factor 1) [135,136]. Association of Smad6 with Smurf-1 alone leads to Runx2 protein degradation, even though Smads 1, 5 and 7 bind to Smurf-1 [137]. miR-503 directly targets Smurf-1, thus preventing Runx2 protein degradation [138]. miR-15 is considered a strong negative regulator of Smurf-1 during osteoblast differentiation [139]. Parallel to the above concept, another ubiquitin ligase, Fbw7α, coordinates degradation of Runx2 in a GSK3β-dependent pathway [140]. Various miRNAs aid in inhibiting Fbw7α ligase but none have been found to target in bones. Protein expression is also reduced by TNF-induced Smurf-1/-2-mediated proteasomal degradation of Runx2 [141]. miR-590 aids in osteoblast differentiation by the direct inhibition of Smad7, which mediates Smurf-2-dependent protein degradation of Runx2 [142]. Individual repression of Smad7 protein is carried out by miR-17, facilitating osteoblast differentiation [143]. Transactivation of Runx2 is inhibited by Skp2, an SCF family E3 ubiquitin ligase that causes proteosome-dependent degradation [144]. CHIP (C-terminus of Hsc70-interacting protein) is another type of E3 ubiquitin ligase that mediates proteasomal degradation of Runx2 and downregulates osteogenesis [145]. miR-764 positively regulates Runx2 expression by the downregulation of CHIP protein [146].

Various other proteins, such as LEF-1 (lymphoid enhancer binding factor 1), TLE proteins, HES-1 (hairy and enhancer of split 1), C/EBPδ (CCAAT/enhancer binding protein δ), Dlx3, Msx2 (Msh homeobox 2), PPARγ (peroxisome proliferator-activated receptors γ), Smad3, Stat1 (signal transducer and activator of transcription 1), Twist target Runx2 and alter its expression [71]. Msx2 is a homeobox protein that interacts with Runx2 and mediates its repression [90]. miR-181 targets Msx2 protein and modulates Runx2 expression indirectly in MC3T3-E1 cells [147]. Stat1 is considered a potent inhibitor of osteoblast differentiation [148] and therefore, its repression mediated by miR-194, enhances Runx2 expression [149]. Another study suggested that miR-29b targets a number of potential inhibitors of Runx2 (HDAC4, TGFβ3, ACVR2A, CTNNBIP1 (catenin Beta Interacting Protein 1), and DUSP2 (dual specificity phosphatase 2) by binding to the 3′ UTR of their mRNAs [150].

Corepressors/activators are certain proteins that modulate a particular gene expression via protein-DNA interaction at the transcription site. As the name suggests, a corepressor has a repressive and inhibitory effect rather than an upregulatory effect. In the case of Runx2, corepressors negatively regulate its expression/activity; whereas coactivators show a positive effect on Runx2. As listed above, it is evident that miRNAs are key regulators that influence Runx2 expression/activity via corepressors and coactivators for osteoblast differentiation (Fig. 2).

4. Therapeutic application of miRNAs in bone-related ailments

Osteoblast differentiation is a tightly regulated process that involves the expression of regulatory genes, osteoblast differentiation marker genes, signaling pathways, and deposition of minerals. As an integral part of the bone remodeling process that keeps the bone sound, it is essential to maintain optimal regulation of osteoblast formation. Many medical conditions like osteoporosis, osteoarthritis, bone cancer, and others are manifested when bone formation is not correctly regulated.

miR-146a/b is found to be a novel molecule for reversing excessive differentiation of osteoclasts by inactivating the NF-κB molecule, an activator of osteoclast precursor cells, which eventually led to reduced joint destruction in arthritic mice [151]. Overexpression or synthetic transfer of miR-145 in chondrocytes can potentially be used for treating cartilage degradation [152]. Many miRNAs that target Runx2 repressors are potential therapeutic candidates: miR-2861 targets HDAC5; miR-29b targets HDAC4 and miR-15b targets E3 ubiquitin ligase; and others [153]. In another study dealing with multiple myeloma bone disease, a cancer of white blood cells with extensive bone resorption, miR-29b downregulates the genes pertaining to osteoclast lineage and NAFTc-1, a master transcription factor of osteoclast [154]. Keeping in mind miRNA's therapeutic potential, the delivery of the molecule to the appropriate site is challenging. The successful use of the miRNA as a “medicine” is being studied seriously in the application of biomaterials and other immunologically accepted compounds for delivery [155,156].

It is possible to transfer miRNAs and target the required tissues using: low-molecular weight protamine (LMWP), a cell-penetrating peptide (CPP) carrier, light-activated conjugate complexes of miRNAs, and many other delivery methods [157,158]. Regulated and prolonged release of miRNAs in the targeted site, instead of immediate or burst release, is another approach pertaining to miRNA-mediated disease treatment. Scaffold-based delivery systems have been used with chitosan as a principle material due to chitosan's ability to release the miRNA in a systemic fashion, negligible cytotoxicity, ease of handling, and high durability [159–163]. The application of PLGA nanoparticles as a therapeutic carrier for the delivery of small and big molecules into the cell has gained popularity due to physiologic compatibility [164]. Hydrogel-based delivery of miR-26 enhances angiogenesis in bone, leading to faster and better vascularization within the bone [165,166]. In a study involving metastatic prostate cancer, the spread of cancer to the bone is inhibited by transfecting the cells with miR-16/atelocollagen complexes [167]. Calvarial bone defects are targeted and

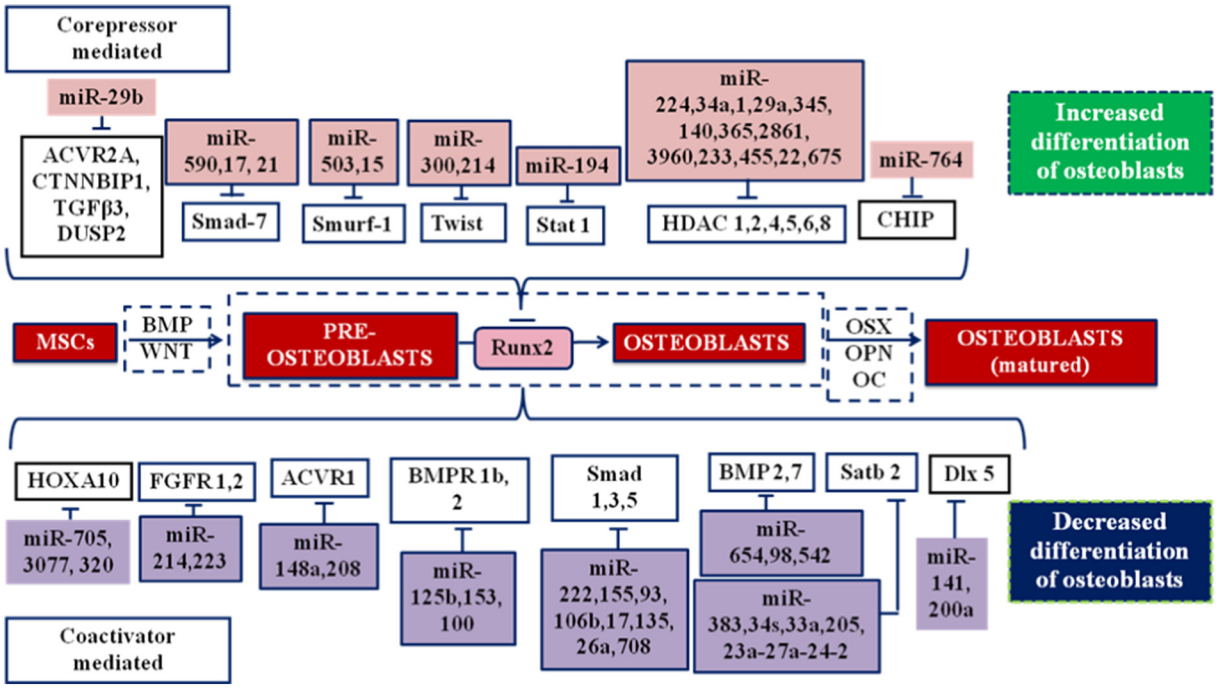

Fig. 2. miRNAs in osteoblast differentiation. miRNAs indirectly targeting Runx2 via its corepressors or coactivators may lead to increased or decreased regulation of osteoblast differentiation, respectively.

treated in a rat model by transfecting anti-miR-31 through poly(glycerol sebacate) scaffolds, which shows great biocompatibility and is also proposed as a therapeutic option for other bone defects [168]. Extensive bone resorption by osteoclasts is monitored and controlled using a DOTAP (N-(1-(2,3-Dioleoyloxy)propyl)-*N*,*N*,*N*-trimethylammonium methyl sulfate)-based liposome conjugated with 8 Asp residues and antagomir-148a that targets the miR-148a [169]. A recent study uses the phytocompound, zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) to promote bone formation by upregulating Runx2 expression and even controlling the negatively regulating miRNAs that target Runx2 [170]. A new porous collagen nano-hydroxyapatite scaffold that targets miR-133 is studied in human MSCs to manipulate upregulation of Runx2 expression [171]. In a study that involved bone metastasis, MDA-MB-231-luc cells transfected with miR-135 and miR-203 downregulate Runx2 expression in tumors, leading to subsequent inhibition of metastasis [48]. Considering these varied methods and their subjects, it can be deduced that bone tissue engineering and biomaterials play a major role in miRNA-dependent osteotherapeutic development [155,172,173].

5. Conclusion

The protagonist of bone formation and remodeling is the master transcription factor, Runx2. The desired effects on osteoblast differentiation can be achieved by regulating Runx2 and its function at various levels of transcription and translation. This review focused on the post-transcriptional regulation of Runx2 orchestrated by miRNAs. miRNAs target the 3′UTR of mRNAs, which either leads to mRNA degradation or its silencing, ultimately hindering its function. However, miRNAs may be either positive or negative regulators of osteoblast differentiation, based on their targeting of either corepressors or coactivators. This review discussed several regulators of bone formation and miRNAs participating in osteogenesis pathways. Exploiting this influential trait of miRNAs is an emerging field of interest in osteotherapeutics. However, the majority of the findings relating miRNAs to bone formation have been obtained through in vitro studies that need to be explored further, and then validated by in vivo studies, to become clinically applicable. An important point to be noted is that a single miRNA may have multiple targets and therefore, before using miRNA as a therapy, it is imperative to understand its mechanisms and targets to avoid repression of genetically vital genes.

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