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Roles of miR-214 in bone physiology and disease

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Abstract: MicroRNAs (miRNAs) are small non-coding RNAs (ncRNAs) that regulate the expression of their target mRNAs post-transcriptionally. Since their discovery, thousands of highly conserved miRNAs have been identified and investigated for their role in human health and diseases. MiR-214 has been increasingly reported to have an association with the regulation of bone metabolism. Reports suggested that miR-214 controls the critical aspects of osteoblasts (bone-forming cells), including their differentiation, proliferation, viability, and migration. Studies have also reported the functional significance of miR-214 in bone diseases and suggested its candidature as a diagnostic and therapeutic target. Further, targeting miR-214 by other ncRNAs, such as linear ncRNAs and circular RNAs, has provided novel insights into treating bone diseases. This review briefly discusses the contemporary findings of the physiological and pathological roles of miR-214 in bone turnover. In addition, we highlight the important ncRNA/mRNA/miR-214 axes influencing osteoblast differentiation that are of therapeutic importance for the treatment of bone-related diseases.

Abbreviations

microRNAs	(miRNAs)
NcRNA	Non-coding RNAs
MSCs	Mesenchymal stem cells
PTH	Parathyroid hormone
BMP2	Bone morphogenetic protein2
TGF-β	Transforming growth factor-beta
COL	Collagen
OC	Osteocalcin
OPN	Osteopontin
AGO	Argonaute
miRISC	miRNA-induced silencing complex
MREs	miRNA response elements
Dnm3os	Dynamin-3 opposite strand
Mgp	Matrix gla protein
Sox9	SRY-box transcription factor 9
TXNIP	Thioredoxin-interacting protein

Introduction

Bone is a highly dynamic organ that remodels throughout life to preserve its strength and mineral equilibrium. It mainly comprises bone-forming osteoblasts and bone-resorbing osteoclasts. The balance between bone formation and resorption remains crucial in maintaining bone remodelling and homeostasis (Katsimbri, 2017). Osteoblasts are differentiated from mesenchymal stem cells (MSCs) in the bone microenvironment via the action of various signalling molecules such as parathyroid hormone (PTH), bone morphogenetic protein2 (BMP2), and transforming growth factor-beta (TGF-β) (Krishnan *et al.*, 2022). They secrete collagen (COL), osteocalcin (OC), osteopontin (OPN), alkaline phosphatase (ALP), and other molecules that are responsible for bone matrix mineralization (Blair *et al.*, 2017). Osteoblast differentiation is a multistep process governed by an integrated gene expression cascade that promotes proliferation and differentiation in sequential order (Ponzetti and Rucci, 2021). Osteoblast differentiation is a very important process in bone remodelling, and any defect in this process can cause improper bone formation leading to diseases like osteoporosis.

MicroRNAs (miRNAs) are endogenous non-coding RNAs (ncRNAs, 22–25 nucleotides) that regulate the target mRNA expression post-transcriptionally. They are implicated in various physiological processes including cell growth, development, apoptosis, hormone signalling, differentiation, metabolism, and pathologies like cancer and cardiovascular diseases (Hanna *et al.*, 2019). Several studies reported across the literature have described the process of miRNA biogenesis

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(Narayanan *et al.*, 2019). miRNA biogenesis begins with synthesizing of the long primary mRNA transcript (pri-miRNA) in the nucleus catalysed by RNA polymerase II (Finnegan and Pasquinelli, 2013). The nuclear microprocessor enzyme, Drosha and Pasha (DGCR8), its cofactor, cleaves the primary transcript into a precursor hairpin loop structure (Ha and Kim, 2014). The Drosha-processed hairpin loop structure with free 5′ and 3′ ends forms the precursor miRNA duplex (pre-miRNA) that is then transported to the cytoplasm via the Exportin protein (Exp), where the cytoplasmic RNase III enzyme Dicer cleaves the hairpin loop. The 5′ guide strand is then preferably selected and loaded onto the Argonaute (AGO) complex forming the miRNA-induced silencing complex (miRISC) (O'Brien *et al.*, 2018). The miRNA-RISC complex specifically targets certain mRNAs via binding to their miRNA response elements (MREs) that are complementary to the seed region on the miRNA. These miRNA-MRE interactions could result in complete degradation of the mRNA or inhibition of their translation, depending on the degree of complementarity between their hybridization (Finnegan and Pasquinelli, 2013; Kim *et al.*, 2017; Pasquinelli, 2012).

Reports suggested that miRNAs could positively or negatively regulate osteoblast differentiation. For instance, Ma *et al.* (2019) demonstrated that miR-96 promotes osteoblast differentiation in mice by activating the Wnt signalling pathway. They identified that miR-96 targets sclerostin (SOST), a Wnt inhibitor, thus enhancing the expression of Wnt markers like Wnt1 and β-catenin. Whereas, Zhang *et al.* (2018) demonstrated that miR-223 inhibits osteoblast differentiation by downregulating the expression of Dehydrogenase/Reductase 3 (DHRS3), a gene involved in retinol metabolism activated during osteoblast differentiation.

Among the multiple miRNAs reported, miR-214 is well studied for its role in bone health and diseases. It has a multitude of gene targets like BMP2, activating transcription factor 4 (ATF4), and SRY-box transcription factor 4 (SOX4), which are responsible for the regulation of osteoblast differentiation. Further, miR-214 has also been implicated in bone diseases like osteoporosis and osteosarcoma (Sun *et al.*, 2018). By targeting key molecules such as Osterix (Osx), ATF4, phosphatase and tensin homolog (Pten), β-catenin, and fibroblast growth factor receptor 1 (FGFR1), miR-214 plays an essential role in senile osteoporosis, as well as in the bone formation and resorption (Yuan *et al.*, 2019).

Extracellular vesicles (EVs) are lipid vesicles that range from 50 nanometers to several thousand nanometers in size. Studies have identified the role of EVs in mediating cell-cell communication by transporting molecules like mRNAs, miRNAs, proteins, and lipids (Munir *et al.*, 2020). EVs can be engineered to carry exogenous miRNAs like miR-214, thus providing a therapeutic strategy against bone diseases (Li *et al.*, 2016). Fig. 1 illustrates the important role of miR-214 in the regulation of several molecules that are critically involved in bone remodelling. This review aims to elaborate the recent advancements in our understanding of miR-214 in the regulation of osteoblast differentiation and how it influences bone health. Further, we discuss the role of lncRNA-miR-214/circ-RNA-mRNA axes reported till date and highlight the candidature of miR-214 to serve as a therapeutic target in treating bone diseases.

Bone homeostasis is maintained by the coupled action of both osteoblasts and osteoclasts. During osteoblast differentiation, mesenchymal stem cells differentiate to form mature osteoblasts. This process is governed by crucial transcription factors like RUNX2 (Runt-related transcription factor 2) and OSX. Because miR-214 negatively regulates FGF, OSX, and ATF4, it has the potential to regulate osteoblast differentiation at different stages of maturation. Osteoclasts are formed from the differentiation of hematopoietic stem cells. The osteoclasts markers M-CSF and RANK stimulate the expression of miR-214 in the early stages of osteoclast differentiation, which inhibits PTEN and enhances NFATc1 expression, thereby stimulating osteoclast differentiation. Further, osteoclast-derived exosomal miR-214 inhibits the differentiation of osteoblasts. Thus, miR-214 acts as a crucial regulator of bone homeostasis by acting on osteoblasts and osteoclasts.

Role of miR-214 in Bone

Multiple studies have reported earlier on the critical role of miR-214 in modulating various biological processes,

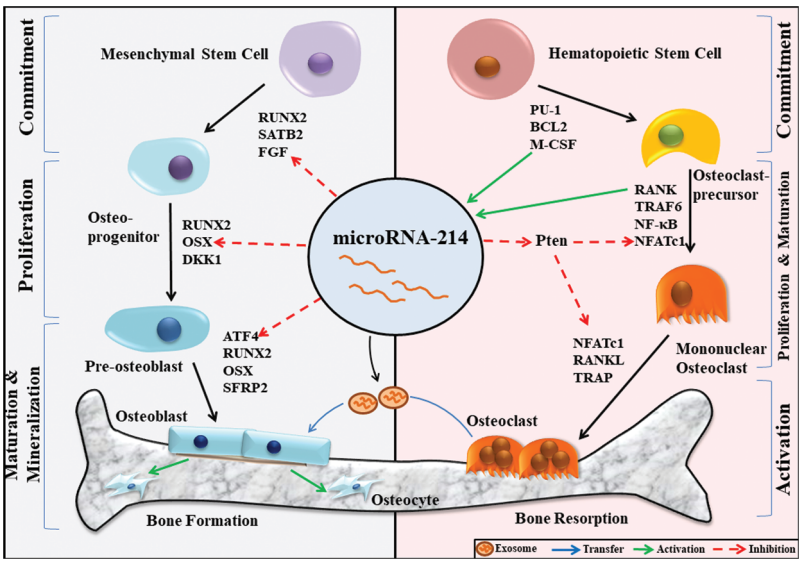

FIGURE 1. MiR-214-mediated regulation of bone homeostasis.

including tissue growth and metabolism, musculoskeletal development etc., and in certain pathological conditions, for instance, osteoporosis, cancer, and others (Amin *et al.*, 2021). MiR-214 is coded by Dnm3os (Dynamin-3 opposite strand), an antisense ncRNA, which was previously found to cause cellular apoptosis in cervical cancer cells (Peng *et al.*, 2017; He *et al.*, 2020). In this section, we have emphasized the involvement of miR-214 in distinct physiological and pathological conditions.

Role of miR-214 in physiological conditions

MiR-214 is essential in stimulating and maintaining various physiological processes, specifically cellular proliferation, followed by tissue formation, which supports growth and development. Numerous studies have revealed the significance of miR-214 in regulating its downstream targets, subsequently triggering bone development (Fig. 2). It plays an imperative role in bone remodelling and chondrocyte differentiation (Iaquinta *et al.*, 2021).

MiR-214 interacts with various intracellular downstream targets, including Col2a1, Mgp and Sox9 in ATDC5 chondrogenic cell lines, PTEN in pre-osteoclasts, ATF4 in human periodontal ligament stem cells, and aids in the development of tissues. It can either positively (indicated in green) or negatively (indicated in red) regulate cellular differentiation.

MiR-214 in bone

In recent years, miR-214 has been found to be involved in regulating intricate bone formation and resorption mechanisms. Yao *et al.* (2017) identified that miR-214 targets ATF4 to suppress the differentiation of human periodontal ligament stem cells into osteoblastic cells, thereby inhibiting bone formation via the miR-214/ATF4 axis. Likewise, in human MSCs (hMSCs), miR-214 overexpression inhibited osteogenesis by targeting β-catenin (Li *et al.*, 2017). A study demonstrated that the downregulation of miR-214 triggered by physical exercise generated-strain on osteoblasts could stimulate osteogenesis *in vivo* and *in vitro*, followed by an upregulation of bone markers including ALP, ATF4 and Osx. However, miR-214 overexpression inhibited the expression of ALP, ATF4, and β-catenin, thus reducing osteogenic differentiation (Yuan *et al.*, 2019). Another study by Li *et al.* (2016) identified that exosomal miR-214 derived from osteoclast induces osteoclast differentiation and suppresses osteoblast differentiation via targeting osteoblast-specific markers including ALP, COL, OPN, OC, and bone sialoprotein (BSP) in the osteoblastic cells, thereby reducing bone formation via increasing resorption. Their results suggested that miR-214 interacts with PTEN and triggers osteoclastogenesis via the Akt/PI3K signalling. Further, osteoclast-specific markers such as tartrate-resistant-acid phosphatase 5a (TRAcP5), semaphorin-4D (Sema4D), and cathepsin K (CTSK) were detected in serum EVs produced by osteoclasts, which could be transmitted to osteoblastic cells to suppress osteogenesis (Li *et al.*, 2016).

Reports have also suggested the participation of miR-214 in the regulation of cartilage formation. Roberto *et al.* (2018) demonstrated an elevated expression of miR-214 in undifferentiated ATDC5 cells (chondrogenic cell line). Increased miR-214 was observed to target Col2a1 (collagen type II alpha 1), Mgp (Matrix gla protein), and Sox9 (SRY-box transcription factor 9). These genes regulated the differentiation of chondrogenic cells into mature chondrocytes, which was, however, restricted by the inhibition of their activities in the presence of miR-214 (Roberto *et al.*, 2018).

Role of miR-214 in pathological conditions

The role of miR-214 in bone physiology is well recognized, as discussed above, but recent studies have shown its participation in regulating different pathological states such as osteoporosis, osteoarthritis, and cancers, including osteosarcoma and Ewing's sarcoma (Fig. 3).

MiR-214 controls several life-threatening diseases that mostly affect bone tissue homeostasis and prominent molecular mechanisms involved in bone growth and development. It significantly inhibits HMGA1 activity in Ewing's sarcoma cells to prevent tumor growth and TRAF3

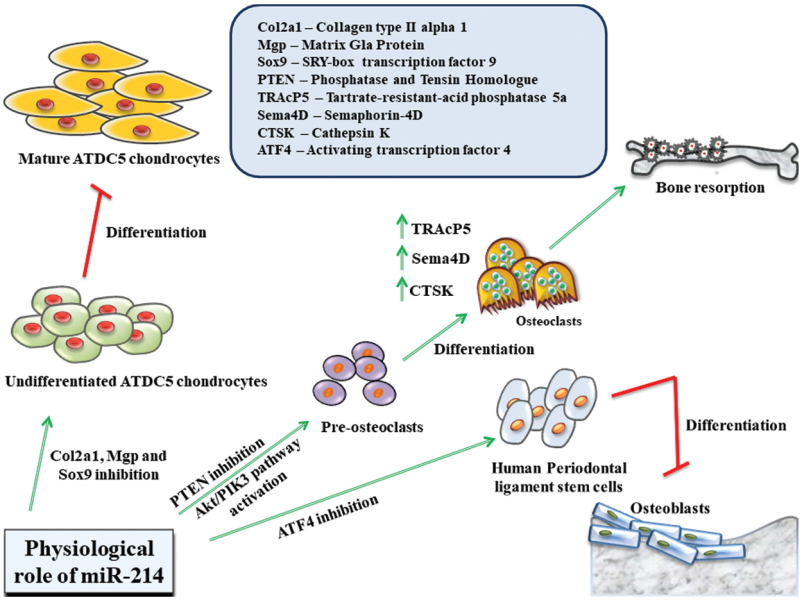

FIGURE 2. Role of miR-214 in regulating the physiological states of the body.

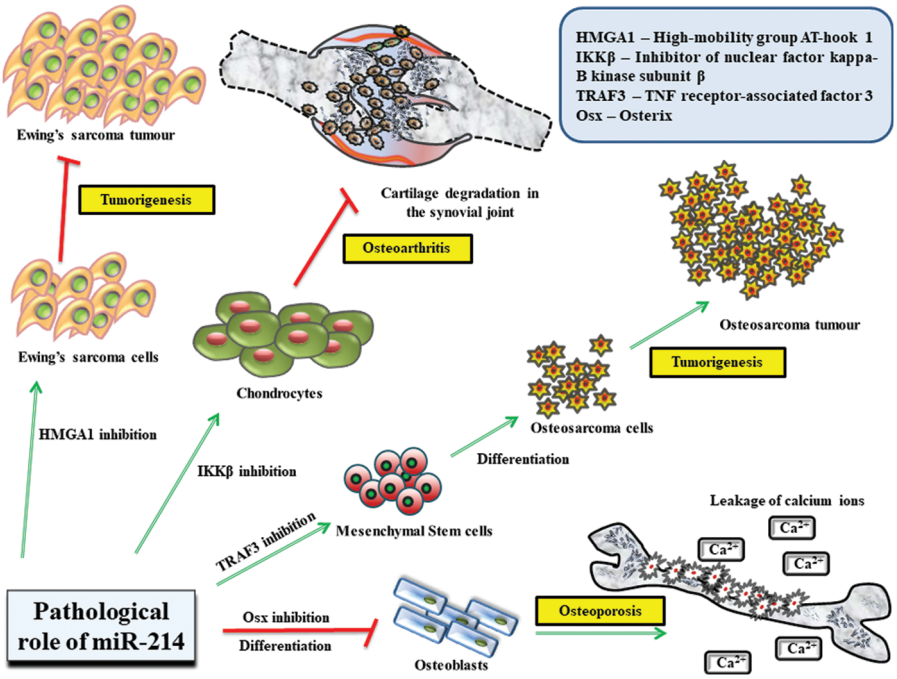

FIGURE 3. Role of miR-214 in regulating the pathological states of the body.

in mesenchymal stem cells from inducing their differentiation into osteosarcoma cells. Additionally, miR-214 obstructs the functions of IKKβ in chondrocytes to impede cartilage degradation. miR-214 also restricts the expression of Osterix in osteoblasts to suppress their differentiation, which culminates in osteoporosis, followed by leakage of Ca^{2+} ions.

MiR-214 in osteoporosis

Osteoporosis is the most prominent and well-known skeletal anomaly that causes fragility in bones leading to enhanced chances of fracture occurrence (Ensrud and Crandall, 2017). miR-214 has been linked to the reduced differentiation of MSCs into osteoblasts and a rapid decline in the mineralization of the bone matrix, thereby triggering osteoporosis (Ge *et al.*, 2017). Mohamad *et al.* (2019) showed the regulatory association between miR-214 and Osx in promoting primary osteoporosis, characterized by reduced bone mass and which mostly affected the trabecular and cortical bones. They identified that the expression profiles of miR-214 and Osx are inversely correlated. Further, they reported a reduction in serum calcium levels and an increase in ALP levels in osteoporotic patients (Sözen *et al.*, 2017; Mohamad *et al.*, 2019). Apart from that, a study found YAP1 (Yes-associated protein 1), a Hippo pathway component, to be a potential target of miR-214 in osteoporotic patients. In particular, YAP1 favours the differentiation of hMSCs into osteocytes. However, miR-214 binds to YAP1 and downregulates its expression, which hinders the differentiation of hMSCs, confirming that miR-214 inhibits YAP1, thereby inducing osteoporosis (Zhong *et al.*, 2021).

MiR-214 in osteoarthritis

Osteoarthritis is the most typical form of bone disability that affects and structurally modifies the whole joint. The structural modification comprises the formation of osteophytes, inflammation in the synovial portions, and degradation of cartilaginous tissues, thereby severely affecting the quality of life (Charlier *et al.*, 2019). A study highlighted the intricate function of miR-214-3p in osteoarthritis. In chondrocytes, the Inhibitor of nuclear factor kappa-B kinase subunit β (IKKβ), an important member of the nuclear factor kappa-B (NF-kB) family, serves as an initiator of the NF-kB pathway. Activation of the NF-kB signalling pathway disrupts processes such as extracellular matrix (ECM) metabolism, followed by the degradation of chondrocytes, thus leading to osteoarthritis. However, miR-214-3p directly targeted and inhibited the activity of IKKβ to exert a chondroprotective effect that aids in preventing the disruption of ECM and chondrocyte apoptosis (Choi *et al.*, 2019; Cao *et al.*, 2021).

miR-214 in osteosarcoma

Osteosarcoma refers to bone cancer that mostly occurs in children, including adolescents, and primarily originates from a single cell in the marrow of long bones, giving rise to a large heterogeneous tumor mass (Brown *et al.*, 2017). Recent research has identified the participation of miR-214 in regulating osteosarcoma (Rehei *et al.*, 2018). miR-214 was found to act as an oncogene and directly regulated the expression of TNF receptor-associated factor 3 (TRAF3) in osteosarcoma cell lines and patient tissue samples. TRAF3, a member of the TRAF family of adapter proteins, is found in almost all bone cells and tissues. When miR-214 was expressed, it reduced the expression levels of TRAF3, thereby leading to osteosarcoma (Rehei *et al.*, 2018). Similarly, Cheng *et al.* (2020) reported that miR-214-3p directly inhibits Fibronectin type III domain-containing protein 5 (FNDC5)/irisin in osteosarcoma cells to promote their migration and invasion. This study indicated that FNDC5 could act as a prospective inhibitor to obstruct the viability and procession of the osteosarcoma cells that are reversed by miR-214-3p (Cheng *et al.*, 2020).

MiR-214 in Ewing's sarcoma

Ewing's sarcoma (EWS) refers to a malignant bone tumor, commonly occurring in the ribs, tibia, pelvis, and femur regions and majorly affects children and young adults. Histologically, EWS belongs to a diverse group of tiny round cell sarcomas, and each cell is morphologically similar to the other (Grünewald *et al.*, 2018). A recent study suggested the regulatory role of miR-214-3p in EWS (de Feo *et al.*, 2022). EWS has two prominent hallmarks, Cluster of differentiation 99 (CD99) and Ewing's sarcoma-friend leukaemia integration 1 (EWS-FLI1). While EWS-FLI1 is an anomalous transcription factor, CD99 is a cell-surface protein molecule (Sen *et al.*, 2018; Manara *et al.*, 2016). It was determined that higher expression levels of CD99 and EWS-FLI1 are exclusively responsible for causing EWS malignancy. Typically, CD99 interacts and binds to EWS-FLI1 to promote malignancy during EWS. In addition, these hallmarks have been described to repress the activity of miR-214-3p, a tumor suppressor in EWS. miR-214-3p has been shown to target HMGA1 (High-mobility group AT-hook 1), which is a small histone protein molecule that engages in tumor growth and metastasis. miR-214-3p overexpression suppressed HMGA1 expression to restrain EWS, but this was opposed by inhibition of miR-214-3p activity in the presence of EWS-FLI1 and CD99, which led to severe EWS malignancy (de Feo *et al.*, 2022).

Together, the findings discussed above illustrate the diverse functions of miR-214 in regulating a wide range of physiological processes in the skeletal system of our body and development at a molecular level.

MiR-214-Mediated Regulation of Osteoblast Differentiation

A repertoire of bone transcription factors, such as Runx2, Osx, ATF4, etc., were reported to regulate bone formation at the transcriptional level (Baek and Kim, 2011). Clinical evidence suggested that miR-214 could regulate bone formation under both physiological and pathological contexts via targeting the key osteogenic factors such as ATF4 (Roberto *et al.*, 2018), Osx (Shi *et al.*, 2013), β-catenin (Cao *et al.*, 2017; Li *et al.*, 2017; Zhu *et al.*, 2017) and so on. This section outlines the molecular processes by which miR-214 influences osteoblasts and the bone microenvironment.

The Wnt/β-catenin pathway governs various crucial processes such as cell fate determination (Boland *et al.*, 2004), embryonic development (Sidrat *et al.*, 2021), immune responses and so on. Any dysregulation in the Wnt/β-catenin pathway has been implicated in bone-related diseases such as osteoporosis (Canalis, 2013). Enhanced activation of this pathway is correlated with increased osteoblastic differentiation of MSCs and decreased miR-214 expression. An increased level of miR-214 was observed to target β-catenin and attenuate the osteoblastic differentiation of MSCs via subduing the Wnt/β-catenin pathway (Li *et al.*, 2017). Furthermore, Cao *et al.* (2017) demonstrated that miR-214 suppresses Wnt/β-catenin signalling by directly targeting β-catenin and inhibits the differentiation of periodontal ligament stem cells into osteoblasts. Likewise, miR-214-3p targeting of β-catenin inhibited the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) (Wang *et al.*, 2019b) and delayed fracture healing (Teng *et al.*, 2018). Additionally, high glucose-induced expression of miR-214-3p was identified to instigate bone loss in Type I diabetic mice via reducing the protein levels of β-catenin (Wang *et al.*, 2019b).

In another study, Wang *et al.* (2013) observed that miR-214 overexpression negatively correlated with the expression of OCN and ALP in osteoblasts. In addition to promoting osteoclastogenesis, the osteoclast-derived exosomal miR-214-3p transferred to osteoblasts has been observed to negatively regulate osteogenesis *in vivo* via targeting the 3′UTR of ATF4 mRNA (Wang *et al.*, 2013; Li *et al.*, 2016). At the same time, Lu *et al.* (2017) observed that miR-214 shields MC3T3-E1 osteoblasts against H_2O_2–induced apoptosis by decreasing reactive oxygen species (ROS) levels via targeting ATF4. In addition, miR-214 expression was observed to be positively correlated with age, suggesting its role as a contributing factor in the prognosis of primary osteoporosis (Mohamad *et al.*, 2019). In another study, Shi *et al.* (2013) concluded that overexpression of miR-214 in C2C12 myoblast cells suppresses osteogenic differentiation by inhibiting endogenous Osx protein synthesis. miR-214 was identified to bind at two sites within the Osx 3′UTR, conducive to downregulating the expression of osteoblast differentiation markers such as ALP, OC and Col1a1 (Shi *et al.*, 2013).

A study by Yang *et al.* (2016) identified that miR-214 directly targets FGFR1 and attenuates the FGFR1/FGF pathway to suppress osteogenesis *in vitro*, while the inhibition of miR-214 using a miR-214 sponge promoted osteoblast differentiation. TNF Receptor Associated Factor 3 (TRAF3) belongs to the TNF Receptor (TNFR) family of proteins crucial for signal transduction and the activation of immune responses. Mir-29b-3p-mediated TRAF3 downregulation has previously been reported to stimulate the progression of triple-negative breast cancer cells. miR-214 targeted TRAF3 to enhance osteoclastogenesis and osteoclastic bone resorption and reduced osteoblast activity in osteolytic breast cancer bone metastasis. Additionally, the transfer of osteoclastic miR-214 to osteoblasts exerted a catabolic effect on bone formation processes (Liu *et al.*, 2017a; Zhang *et al.*, 2019). Guo *et al.* (2017) reported a negative regulation of osteogenic differentiation of BMSCs by miR-214 via direct targeting of JNK and p38 MAPK pathways. Another study by Liu *et al.* (2017b) identified that miR-214 targets Baculoviral IAP repeat containing 7 (BIRC7), pivotal in anti-apoptosis and maintenance of osteoblast activation, and inhibits the differentiation of human osteoblasts via promoting STAT1 expression. Additionally, miR-214 inhibited SOX4 expression in osteoblasts, thereby inducing fragility fracture and regulating fracture healing (Xin *et al.*, 2020).

JNK, FGF, p38 MAPK, and Wnt/β-catenin pathways are some key signaling pathways that influence the proliferation and differentiation of osteoblasts. Although predominantly secreted by osteoclastic EVs, several studies have reported the functional importance of miR-214 in regulating these pathways in osteoblasts and osteoclasts, suggesting that miR-214-mediated crosstalk between osteoblasts and osteoclasts could be a crucial factor in the regulation of bone microenvironment. The mechanisms behind the

crosstalk between these two functionally contrasting cell groups need to be elucidated for a better understanding of the therapeutic potential of miR-214 as a biomarker in diagnosing and treating bone degenerative diseases.

Other Non-Coding RNAs Targeting miR-214 in Osteoblasts

As discussed in the previous sections, miR-214 plays an important role in health and disease, thus making it a suitable biomarker and therapeutic target for bone-related diseases. Since RNA-based therapeutics are gaining interest, ncRNAs are currently employed as therapeutics for various diseases like cancer, cardiovascular diseases, and many more (Wang *et al.*, 2019c; Huang *et al.*, 2020a). ncRNAs are well-studied for their regulatory role in gene expression. Among these ncRNAs, lncRNAs and circRNAs are increasingly reported to regulate gene expression by acting as miRNA sponges (Wang *et al.*, 2019a; Zhu *et al.*, 2020). In this section, we discuss those ncRNAs that target miR-214 and regulate osteoblast differentiation.

Long non-coding RNAs targeting miR-214

LncRNAs are a class of ncRNAs that are greater than 200 nucleotides in length and perform several functions, including chromatin remodelling, epigenetic modifications on DNA, serving as protein scaffolds, and acting as miRNA sponges (Paraskevopoulou and Hatzigeorgiou, 2016). Over the past years, several lncRNAs have been identified to promote or suppress osteoblast differentiation. While the lncRNAs MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) and *H19* (imprinted maternally expressed transcript) promoted osteoblast differentiation (Xiao *et al.*, 2017; Liang *et al.*, 2016), lncRNAs HOTAIR (HOX transcript antisense RNA) and DANCR (differentiation antagonizing non-protein-coding RNA) inhibited osteoblast differentiation (Wei *et al.*, 2017; Zhu and Xu, 2013). Several lncRNAs that target miR-214 have been reported to date. For instance, lncRNA KCNQ1OT1 sponged miR-214 and upregulated BMP2 expression, thereby stimulating the osteogenic differentiation of BMSCs (Wang *et al.*, 2019a). Similarly, lncRNA XIST (X-inactive specific transcript) stimulated the osteogenic differentiation of periodontal ligament stem cells (PDLSCs) by sponging miR-214-3p (Feng *et al.*, 2020). Stromal cell-derived factor-1 (SDF1) regulated osteoblast differentiation by stimulating BMP2 expression (Hosogane *et al.*, 2010). A recent study demonstrated that lncRNA H19 targets miR-214-5p upon SDF1 exposure and increases BMP2 expression, hence promoting the osteoblast differentiation of BMSCs (He *et al.*, 2021).

Li *et al.* (2020) demonstrated that lncRNA LOC100506178 sponged miR-214-5p and promoted BMP2 expression, subsequently favoring the differentiation of BMSCs towards osteogenic lineage. Furthermore, they identified that lncRNA LOC100506178 overexpression in BMSCs and transplantation of the same in nude mice reverses the inhibitory effect of miR-214-5p and promoted ectopic bone formation *in vivo* (Li *et al.*, 2020). While miR-214 was often reported to negatively regulate osteoblast differentiation, Yang *et al.* (2021) identified that miR-214 could promote osteoblast differentiation by targeting thioredoxin-interacting protein (TXNIP). TXNIP is responsible for bone loss during osteoporosis, hence using a rat osteoporosis model, they found that lncRNA MEG3, which sponges miR-214, was upregulated in the osteoporosis condition. Further, siRNA-mediated silencing of lncRNA MEG3 or miR-214 overexpression led to increased expression of OPG, thereby enhancing osteoblast differentiation. Thus, lncRNA MEG3/miR-214/TXNIP is important in understanding and treating osteoporosis (Yang *et al.*, 2021).

Long-intervening ncRNAs (lincRNAs) are a unique group of lncRNAs without protein-coding ability. LincRNAs have been recently identified and are known to play a role in several diseases, including osteosarcoma (Deniz and Erman, 2017). A recent study revealed that LINC00657 induced osteogenic differentiation of BMSCs via targeting miR-214-3p, and upregulating BMP2 expression (Li *et al.*, 2022). Since lincRNAs are only gaining interest recently, we speculate the involvement of many novel lincRNAs that would be important regulators of osteoblast differentiation.

Circular RNAs targeting miR-214

CircRNAs are single-stranded, ncRNA molecules with persistent loop configuration and are known to regulate gene expression. They are highly stable molecules with high tissue specificity, making them ideal for therapeutics. Although circRNAs have been discovered for a long time, their role in human physiology and disease has only recently been investigated (Verduci *et al.*, 2021). CircRNAs can sponge miRNAs and regulate osteoblast differentiation. For instance, circ_0006873, circ_FAT1, and circ_0066523 promoted osteoblast differentiation by sponging their respective target miRNAs (Ye *et al.*, 2021; Xin *et al.*, 2021; Lv *et al.*, 2022).

Another study in a mice osteoporosis model demonstrated that circ_ITCH1 sponges miR-214 and upregulates the expression of YAP1. Higher expression of YAP1 corresponds to enhanced osteoblast differentiation, making circ_ITCH1 an important biomarker and a therapeutic target for the treatment of osteoporosis (Zhong *et al.*, 2021). Runx3 is a positive mediator of osteoblast differentiation and a direct target of miR-214 (Wang *et al.*, 2017). Circ_33287 was observed to sponge miR-214-3p and protected Runx3 from miR-214-3p mediated inhibition, thereby promoting osteogenic differentiation in maxillary sinus membrane stem cells. Further, overexpression of circ_33287 and miR-214-3p inhibition was identified to stimulate ectopic bone formation *in vivo*. Hence, circ_33287/miR-214-3p/Runx3 axis presents a novel molecular approach for bone regeneration in the posterior maxilla (Peng *et al.*, 2019). Studies have demonstrated that circRNAs could regulate the proliferative and invasive capacity of osteosarcoma cells (Ji *et al.*, 2020). Mao *et al.* (2021) identified the competing endogenous RNA (ceRNA) role of circ_XPR1 in osteosarcoma cells. They reported that circ_XPR1 sponges miR-214-5p and promotes the proliferative capacity of osteosarcoma cells via regulating the expression of DEAD-Box Helicase 5 (DDX5), a target of miR-214-5p. Their findings suggested that circ_XPR1/miR-

TABLE 1

ncRNA/miR-214/mRNA axis in bone diseases

Axis name	Condition	Effect	Reference
lncRNA HAGLR/miR-214-3p/BMP2	Tibial fractures	Promoted bone healing by enhancing the expression of OPG and ALP	Chen and Yang (2021)
lncRNA TUG1/miR-214/BMP2	Maxillary fractures	Promoted proliferation and differentiation of osteoblasts	Yao *et al.* (2022)
lncRNA MALAT1/miR-214/ATF4	Steroid-induced avascular necrosis of the femoral head (SANFH)	Promoted osteoblast differentiation	Huang *et al.* (2020b)
circ_0001843/miR-214/TAFA5	Osteoporosis	Reduced proliferation and differentiation of osteoblasts	Zhu *et al.* (2020)
circ_ITCH1/miR-214/YAP1		Promoted osteoblast differentiation	Zhong *et al.* (2021)
LINC01535/miR-214-3p/KCNC4	Osteosarcoma	Promoted proliferation and invasion	Yao *et al.* (2020)
LINC00612/miR-214-5p/SOX4		Promoted invasion, proliferation and EMT	Zhou *et al.* (2020)
circ_0016347/miR-214/Caspase1		Promoted proliferation and metastasis	Jin *et al.* (2017)

214-5p/DDX5 axis may serve as an important therapeutic target for osteosarcoma (Mao *et al.*, 2021). Table 1 summarizes the involvement of ncRNA/mir-214/mRNA axes in bone-related diseases.

The above-discussed reports highlighted the emerging role of ncRNAs targeting miR-214 in treating various bone-related disorders. However, many of these ncRNAs are still in the preclinical stage due to the challenges in developing RNA-based therapeutics. Challenges such as degradation by RNases and the inability to cross cell membranes are a few concerns that have to be addressed for the efficient development of ncRNA-based therapeutics.

Conclusions

MiRNAs have emerged to be pivotal in the post-transcriptional regulation of gene expression. In particular, miR-214 is extensively reported for its role in human health and diseases, especially in the musculoskeletal system. In the current review, we explained the function of miR-214 in bone biology and its effect on osteoblast differentiation. Studies reported that miR-214 regulates several aspects of osteoblasts, including their differentiation, proliferation, viability, and migration. Further, miR-214 has a key role in bone diseases like osteoporosis and osteosarcoma. The ability of miR-214 to regulate different cellular processes is due to its multitude of gene targets, thus making miR-214 a potential biomarker and therapeutic target. NcRNA-based therapeutics specifically aimed at miR-214 may tend to cure or alleviate the effects of diseases like osteoporosis. However, challenges associated with RNA-based therapeutics have to be addressed. Nevertheless, extensive studies on miR-214 might help us devise better therapeutic strategies to combat bone-related diseases.

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