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REVIEW

MicroRNAs as regulators of bone homeostasis and bone metastasis

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MicroRNAs (miRNAs) are short, endogenous RNAs that have essential roles in regulating gene expression through the disruption of target genes. The miRNA-induced suppression can occur through Argonaute-mediated cleavage of target mRNAs or by translational inhibition. System-wide studies have underscored the integral role that miRNAs play in regulating the expression of essential genes within bone marrow stromal cells. The miRNA expression has been shown to enhance or inhibit cell differentiation and activity, and elucidating miRNA targets within bone marrow cells has revealed novel regulations during normal bone development. Importantly, multiple studies have shown that miRNA misexpression mediates the progression of bone-related pathologies, including osteopetrosis and osteoporosis, as well as the development and progression of osteosarcoma. Furthermore, recent studies have detailed the capacity for miRNAs to influence bone metastasis from a number of primary carcinomas. Taken together, these findings reveal the significant clinical potential for miRNAs to regulate bone homeostasis, as well as to mediate bone-related pathologies.

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MiRNA Biogenesis and Function

The past decade has seen a torrent of novel research into the post-translational regulation of genes via microRNA-mediated suppression. The microRNAs (miRNAs) are a class of ~22-nucleotide-long RNAs that repress gene expression through complementary binding to sites in the 3′-untranslated region (UTR) of target mRNAs.[1] Mature miRNAs are generated by the sequential cleavage of longer precursor transcripts, or pri-miRNAs, that are typically transcribed from intragenic or intergenic regions by RNA polymerase II.[2] The initial cleavage step, mediated by Drosha and the DGCR8 complex, occurs in the nucleus and produces a shortened (70–100 nucleotides) pre-miRNA hairpin. Pre-miRNAs are exported from the nucleus by Exportin 5, followed by a second cleavage by the ribonuclease Dicer that produces a double-stranded, ~18–25-nucleotide-long mature miRNA. One miRNA strand will then combine with Argonaute (AGO2) proteins to produce an RNA-induced silencing complex, allowing for directed pairing with target mRNAs.[1]

The miRNA targeting of mRNAs is regulated through binding of the 2–8 nucleotides from the 5′ end of the miRNA, a region known as the 'seed sequence', to complementary regions within the target 3′-UTR.[1,3] There are currently two validated mechanisms for miRNA-mediated inhibition of target genes: mRNA degradation or translational silencing. Cleavage of target mRNAs is most commonly observed in situations where the miRNA and target mRNA exhibit complete complementarity. In these rare situations it is possible to observe the cleavage and subsequent degradation of a bound mRNA target.[3] Conversely, miRNA–mRNA pairings that feature imperfect complementarity result in translational inhibition in the absence of target cleavage.[3,4] Thus, even in the absence of perfect binding, the association between a miRNA and a target 3′-UTR still results in the suppression of a target protein. Because of the relatively short length of the seed sequence, each miRNA is capable of binding to hundreds of mRNAs. Although this presents a significant hurdle, recent advances in computational and biochemical methods have aided target prediction, opening the way for studies into miRNA-mediated regulation of target genes.[3]

MiRNA Regulation within Bone Marrow Cells

As important regulators of gene expression, miRNAs themselves are subject to complex control. Initial modulation can occur either through regulation of pri-miRNA transcription or through controlling of processing into a mature strand via alterations in the activity of Drosha or Dicer.[2] Within bone homeostasis, alterations in miRNA processing have been shown to have a profound effect on the function of bone marrow cells. For example, *in vivo* deletion of *Dicer* in osteoprogenitors, osteoblasts and chondrocytes, using a *Col1a1* promoter-driven

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Cre recombinase, resulted in severe skeletal deformities in fetal mice.[5] These mice exhibited a misproportioned cartilage skeleton at E14.5 and displayed a reduction in mineralized tissue formation due to defects in osteoblast maturation. When dicer was ablated by *Osteocalcin*-Cre, eliminating miRNA processing in mature osteoblasts, the mice were viable but exhibited delayed bone development that corresponded with reduced osteoblast numbers.[5] Similarly, *Dicer* knockout in chondrocytes, using *Col2a1*-Cre mice, modulated proliferation and differentiation.[6] These transgenic mice presented with significant skeletal defects that were associated with the differentiation of cells into postmitotic hypertrophic chondrocytes featuring decreased proliferation. Subsequent analysis has revealed the expression of several hundred miRNAs within chondrocytes under physiological conditions.

Osteoclast activity has been similarly tied to proper miRNA expression. Osteoclast-specific *Dicer* knockout, utilizing *CD11b*-Cre transgenic mice, exhibits defective osteoclastogenesis, with a reduction in multinuclear osteoclasts and increased bone mass.[7] Cultured bone marrow from these mice was incapable of producing osteoclasts *ex vivo*, further confirming the defect in osteoclast maturation.[8,9] Importantly, this defect was not confined to Dicer; knockdown of DGCR8 and Ago2 using siRNA similarly resulted in decreased osteoclast differentiation and bone resorption, and osteoclast-specific *DGCR8* knockout mice display impaired bone development.[7,10] Taken together, these results reveal the necessity for proper miRNA regulation during the differentiation and maintenance of multiple essential cell types required for bone homeostasis.

A Central Role for MiRNAs in Bone Homeostasis

The maintenance of homeostasis within the bone microenvironment depends on the careful orchestration of multiple cell types, each of which maintains precise expression of a multitude of genes that regulate cellular activity.[11,12] Importantly, the molecular pathways that regulate differentiation of bone cells are coordinated through cross-talks among neighboring stromal cells.[13] Thus, it is not surprising that general disruptions within the miRNA biogenesis machinery described above would have dramatic impacts on physiological bone remodeling. Interestingly, a number of recent studies have revealed the capacity for individual miRNAs to direct the differentiation and activity of cells residing in the bone microenvironment (**Figure 1a**).

Chondrocytes, essential regulators of longitudinal bone growth, have been shown to be intricately regulated by a series of miRNAs. MiR-140, a positive regulator of chondrogenesis through the inhibition of *HDAC4* and *Dnpep*, contributes to craniofacial development and endochondral bone formation.[14–16] Consistent with this, mice lacking miR-140 display accelerated bone formation at embryonic and neonatal stages of development.[15] Conversely, miR-199a* and miR-145 have been shown to negatively regulate chondrocyte differentiation; miR-199a* downregulates the expression of Smad1,[17] whereas miR-145 targets *Sox9*, a key transcription factor involved in chondrogenesis.[18] Thus, miRNAs can serve both negative and positive regulatory roles during chondrocyte differentiation.

Similar miRNA-based regulation has been observed during osteoblast differentiation and activity. MiR-138 inhibits osteogenesis by directly targeting focal adhesion kinase, a critical factor in osteoblast differentiation, leading to attenuated bone formation *in vivo*.[19] Importantly, when human mesenchymal stem cells were treated with anti-miR-138 oligonucleotides, there was a significant increase in osteogenic capacity and bone formation. The miR-23a/-27a/24-2 cluster is similarly tied to osteogenesis through a Runx2 regulatory loop. Expression of the miRNA cluster can be transcriptionally repressed by a Runx2 binding site in the cluster's promoter, whereas miR-23a is capable of directly repressing *Runx2* expression.[20] Thus, Runx2-mediated inhibition of the

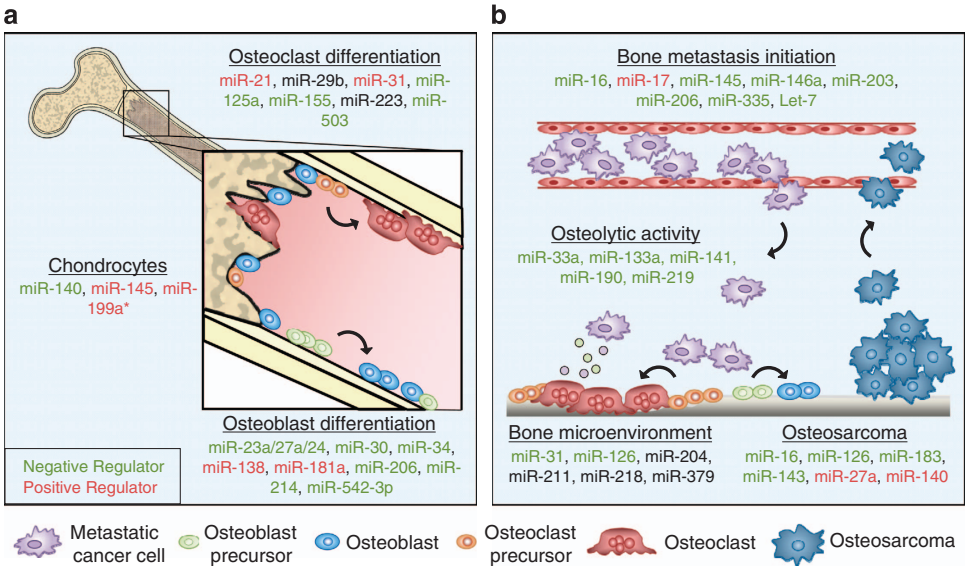

Figure 1 The miRNA involvement in bone homeostasis and bone metastasis. (**a**) The miRNA-mediated regulation of osteoclast, osteoblast and chondrocyte differentiation and activity is essential for proper bone development and maintenance. Known positive (red) and negative (green) regulators are indicated. (**b**) Bone metastasis migration, invasion and homing to the bone microenvironment are controlled by multiple miRNAs. In addition, miRNAs regulate bone metastasis activity, including the tumor–stromal cross-talk that mediates osteolysis. Finally, the development of osteosarcoma depends on proper regulation of miRNAs that can enhance or inhibit tumor progression.

miR-23a/-27a/24-2 cluster initiates a positive feedback loop. In addition to increased expression of Runx2, the inhibition of miR-23a/-27a/24-2 also derepresses *SATB2*, an additional activator of osteogenesis, allowing for progression of the cell through osteoblast differentiation.[20] The miR-206 expression was shown to inhibit osteoblast development, whereas miR-206 knockdown promoted differentiation.[21] Many additional miRNAs have been shown to play a role in osteogenesis (see **Figure 1a** and **Table 1**).

Osteoclast differentiation likewise involves the temporally organized coordination of multiple transcription factors and regulatory proteins. Unsurprisingly, the expression of many of these proteins is modulated by miRNA-mediated silencing. MiR-223 has been well studied in osteoclastogenesis, with conflicting studies indicating that miR-223 expression is capable of simultaneously enhancing and suppressing osteoclast differentiation.[7,22] Similarly, the miR-29 family has been shown to play a dichotomous role during osteoclastogenesis. In one study, ectopic miR-29b expression inhibited osteoclast activity,[23] whereas a more recent report found that miR-29b was able to promote osteoclastogenesis by directly targeting *Cdc42* and *Srgap2*, and knockdown of miR-29 in pre-osteoclasts inhibited differentiation.[24] Additional studies have uncovered miRNAs with more easily defined roles during osteoclast differentiation. Mizoguchi *et al.*[25] found that miR-31, which is highly upregulated during osteoclast development, is essential for proper differentiation. Similarly, miR-21 has been shown to positively regulate osteoclastogenesis by targeting the c-Fos inhibitor *PDCD4*.[9] Conversely, *RANK* targeting by miR-503 inhibited osteoclastogenesis, whereas silencing miR-503 enhanced *in vivo* bone resorption.[26] MiR-125a was also shown to inhibit osteoclast differentiation by suppressing *TRAF6* expression,[27] whereas miR-155 was shown to regulate cell-fate commitment

Table 1 The miRNA involvement in bone homeostasis

MiRNA biogenesis	*Regulatory function*	*Activity*	*Reference*
Dicer	Essential for development	Knockout inhibits osteoblast, osteoclast, chondrocyte development	5–9
DGCR8	Essential for development	Knockout inhibits osteoclast development	7, 10
Ago2	Essential for development	Knockout inhibits osteoclast development	7
Osteoclast			
miR-223	Activates and/or inhibits	Positive and negative regulator of differentiation	7, 22
miR-29b	Activates and/or inhibits	Activates and/or inhibits differentiation, targets *Cdc42, Srgap2*	23, 24
miR-31	Essential for development	Essential for differentiation	25
miR-21	Promotes	Promotes differentiation, targets *PDCD4*	9
miR-148a	Promotes	Promotes differentiation, targets *MAFB*	88
miR-125a	Inhibits	Inhibits differentiation, targets *TRAF6*	27
miR-155	Inhibits	Inhibits differentiation, targets *SOCS1, MITF*	28, 29
miR-503	Inhibits	Inhibits differentiation, targets RANK	26
miR-146a	Inhibits	Inhibits differentiation, protects joint destruction during Collagen-induced arthritis	89
Osteoblast			
miR-138	Inhibits	Inhibits osteoblast differentiation, targets *FAK*	19
miR-23a/27a/24–2	Inhibits	Inhibits apoptosis, regulates *FAK, Runx2, SATB2*	20, 82
miR-20a	Promotes	Promotes differentiation, targets *PPARγ, Bambi, Crim1*	90
miR-181a	Promotes	Promotes osteoblast differentiation, targets *Tgfbi*	86
miR-335–5p	Promotes	Promotes differentiation, targets *DKK1*	91
miR-15b	Promotes	Promotes differentiation, targets *Smurf1*	92
miR-17∼92	Promotes	Promotes proliferation, activity in mouse model	93
miR-322	Promotes	Promotes differentiation, targets *Tob2*	94
miR-29a	Promotes	Promotes differentiation, targets *Osteonectin, Dkk1, Kremen2, sFRP2*	36, 95, 96
miR-34	Inhibits	Inhibits differentiation, targets *SATB2, Runx2,* Notch pathway	30–32, 84
miR-143	Inhibits	Inhibits differentiation, targets *Osterix*	97
miR-155	Inhibits	Regulates TNF-α inhibition of osteoblast differentiation, targets *SOCS1*	98
miR-93	Inhibits	Osteoblast mineralization, targets Sp7	99
miR-182	Inhibits	Inhibits differentiation, targets *FoxO1*	100
miR-764–5p	Inhibits	Inhibits differentiation, targets *CHIP/STUB1*	101
miR-208	Inhibits	Inhibits differentiation, targets *Ets1*	102
miR-206	Inhibits	Inhibits differentiation, targets Cx43	21
miR-30	Inhibits	Inhibits differentiation, targets *Smad1, Runx2*	83
miR-214	Inhibits	Inhibits activity and bone formation, targets *ATF4, Osterix*	85, 103
miR-542–3p	Inhibits	Inhibits differentiation, targets *BMP7*	87
miR-141, miR-200a	Inhibits	Inhibits differentiation, target *Dlx5*	104
miR-100	Inhibits	Inhibits differentiation, targets *BMPR2*	105
miR-17–5p, miR-106	Inhibits	Inhibits differentiation, targets *BMP2*	106
Chondrocytes			
miR-140	Essential for activity	Essential for proper activity, targets *HDAC4, Dnpep*	14–16
miR-34a	Inhibits	Inhibits differentiation, regulates apoptosis	107, 108
miR-199a*	Inhibits	Inhibits differentiation, targets *Smad1*	17
miR-145	Inhibits	Inhibits differentiation, targets *Sox9*	18
miR-17∼92	Essential for development	Loss induces microcephaly and other skeletal defects in patients	33

within macrophages through the downregulation of *SOCS1* and *MITF*, effectively inhibiting differentiation through an osteoclast lineage.[28,29]

It is important to recognize that the regulation of bone homeostasis depends on carefully orchestrated cross-talk between bone marrow cells. MiR-34c is an essential regulator of Notch signaling in osteoblasts, directly targeting *Notch1*, *Notch2* and *Jagged1*, as well as *Satb2* and *Runx2*.[30] Interestingly, miR-34c has been shown to function with both cell-autonomous and non-cell-autonomous activities. Although upregulation of miR-34c inhibits osteoblast differentiation by decreasing *Satb2* and *Runx2*, the inhibition of Notch signaling might have a role in regulating the RANKL/OPG ratio, leading to increased osteoclast differentiation.[30,31] Thus, an individual miRNA is capable of simultaneously regulating multiple bone marrow cells to maintain homeostasis.

MiRNA Regulation of Other Bone-Related Pathologies

Given the profound effect that miRNA misregulation can have on bone marrow cell differentiation, it is perhaps not surprising that miRNAs have also been shown to regulate a number of bone-related pathologies. In addition to the broad skeletal defects seen from the unbiased ablation of miRNA biogenesis, specific miRNAs have been found to have a role in pathologies related to impaired osteoblast and osteoclast activity. For example, the miR-34 family of miRNAs serves as key regulators of osteoblast maturation. Wei *et al.*[32] found that miR-34 could inhibit osteoblast terminal differentiation through the inhibition of *SATB2*, and could additionally inhibit proliferation of mature osteoblasts by inhibiting *Cyclin D1*, *CDK4* and *CDK6*. These effects were confirmed in the pronounced skeletal defects seen in transgenic mice featuring ectopic miR-34c under the control of *Col1a1*. Similarly, the miR-17∼92 cluster, comprising six well-studied miRNAs, has been tied to skeletal defects in humans. De Pontual *et al.*[33] uncovered hemizygous deletions of the miRNA cluster in patients with microcephaly, featuring shortened stature and digital abnormalities, and miR-17∼92 heterozygous mice phenocopy the human developmental features.

In addition, a number of miRNAs have been shown to function during osteoporosis. High-throughput screening revealed broad changes in miRNA expression during age-related osteoporosis; examination of the overlap between mesenchymal stem cells and bone tissue in a mouse model of osteoporosis revealed the differential expression of 8 upregulated and 30 downregulated miRNAs.[34] MiR-2861 is normally transcribed during BMP2-induced osteogenesis, and inhibition results in the derepression of histone deacetylase 5, itself an enhancer of Runx2 degradation, leading to decreased bone formation.[35] Importantly, loss of miR-2861 activity was found to be responsible for osteoporosis in two adolescent patients. Interestingly, miR-29a, which had been previously shown to suppress Osteonectin during osteoblastogenesis,[36] was found to protect against glucocorticoid-induced bone loss, a frequent pathological repercussion that leads to decreased osteoblast survival and increased osteoclast activity.[37] Forced expression of miR-29a attenuated the effects of glucocorticoid treatment, increasing trabecular bone mass and decreasing cortical bone porosity.

MiRNA Expression Influences Growth and Progression of Osteosarcoma

Osteosarcoma, the most common primary sarcoma of bone, remains the leading cause of cancer-related death in adolescents.[38,39] Although questions remain as to the molecular basis of osteosarcoma, patients frequently present with high-grade tumors seemingly of osteoblastic or fibroblastic origin. The miRNA signatures within osteosarcoma have been shown to associate with pathological features, whereas specific miRNAs can regulate tumor progression and metastasis[40] (**Figure 1b** and **Table 2**). For example, miR-16 was found to function as a tumor suppressor in human osteosarcoma cell lines, decreasing tumor volume and enhancing apoptosis. Similarly, miR-126 is frequently downregulated in osteosarcoma, and ectopic expression inhibited invasion and induced apoptosis in osteosarcoma cells.[41] MiR-183 and miR-143, which target *Ezrin* and *MMP-13*, respectively, can similarly inhibit osteosarcoma migration and invasion.[42,43] Importantly, ectopic expression of miR-143 was able to suppress lung metastasis in a mouse model of human osteosarcoma.[43] Conversely, miR-27a enhanced migration and invasion *in vitro* while simultaneously increasing pulmonary and bone metastasis after intravenous inoculation.[40] MiR-21 was similarly shown to function as a driver of osteosarcoma migration and invasion, and suppression of miR-21 reduced motility in osteosarcoma cell lines by direct targeting of *RECK*.[44]

In addition to a direct role in osteosarcoma pathology, miRNAs might also serve as biomarkers for disease progression and response to chemotherapy. A number of miRNAs, including miR-140 and miR-215, have been shown to regulate osteosarcoma chemoresistance.[45,46] Ectopic miR-215 protected osteosarcoma and colon cancer cell lines from methotrexate (MTX) and Tomudex, whereas miR-140 overexpression desensitized osteosarcoma cells to MTX and 5-fluorouracil. More recently, miRNA expression analysis of chemoresistant patient samples and cell lines revealed a miRNA signature consisting of miR-92a, -99b, -132, -193a-5p and -422a that was capable of predicting response to ifosfamide, whereas miR-33a reduced cisplatin-induced apoptosis.[47,48]

Regulation of Multiple Myeloma via miRNAs

The progression of multiple myeloma (MM) has also been shown to require the careful coordination of miRNA regulatory pathways (**Table 2**). MiR-29b has been shown to induce apoptosis in MM cells through inhibition of *MCL1*,[49] and ectopic expression of miR-29b can inhibit DNA methyltransferase activity in human MM cells by directly targeting *DNMT3A* and *DNMT3B*.[50] Ectopic expression of miR-29b attenuated global DNA methylation, inhibited cell-cycle progression and reduced tumor growth in an *in vivo* model of MM. A follow-up study uncovered miR-29b-mediated upregulation of *SOCS1* due to reduced promoter methylation, leading to increased adhesion of MM cells to bone marrow stromal cells and decreasing migration *in vitro*.[51] Similarly, miR-34a was found to decrease *in vivo* tumor growth of human MM cells, potentially through the regulation of *BCL2*, *CDK6* and *NOTCH1*.[52] MiR-15a and miR-16, which are frequently suppressed in MM, inhibit proliferation and growth of MM both *in vitro* and *in vivo*.[53] In addition, both miRNAs were shown to directly regulate *VEGF-A* expression during MM progression, inhibiting angiogenesis and decreasing

Table 2 The miRNA involvement in bone metastasis and osteosarcoma

Tumor seeding	*Activity*	*Validated targets*	*Model*	*Reference*
Let-7g	Inhibits migration, bone metastasis	HMGA2	Human breast cancer cell lines, intracardiac injected bone metastasis in mice	58
miR-17	Enhances migration and metastasis		Human breast cancer cell lines, spontaneous bone metastasis in mice	59
miR-335	Inhibits invasion, bone metastasis	Sox4, Tenascin C	Human breast cancer cell lines, intracardiac injected bone metastasis in mice	60, 61
miR-206	Inhibits invasion, dissemination		Human breast cancer cell lines, intracardiac injected bone metastasis in mice	60
miR-203	Inhibits bone metastasis	Zeb2, Bmi, Survivn, Runx2	Human prostate cancer cell lines, intracardiac injected bone metastasis in mice	67
miR-16	Inhibits bone metastasis		Human prostate cancer cell lines, systemic miR-16 inhibits bone metastasis	68
miR-146a	Inhibits proliferation and adhesion to endothelial cells,	ROCK	Human prostate cancer PC3 cells *in vitro*	69
miR-145	Inhibits migration, seeding	HEF1	Human prostate cancer PC3 cells *in vitro*	70, 71
Bone marrow microenvironment				
miR-218	Osteomimicry in breast cancer cells	SOST, DKK2, SFRP2	Human breast cancer cells *in vitro*	62
miR-126	Knockdown enhances bone metastasis, inhibits endothelial cell recruitment in lung	IGFBP2, PITPNC1, MERTK	Human breast cancer cell lines, intracardiac injected bone metastasis in mice	65
miR-31	Regression of established metastasis	ITGA5, RDX, RhoA	Human breast cancer cell lines, intracardiac injected bone metastasis in mice	66
miR-33a, miR-133a, miR-141, miR-190, miR-219	Inhibits osteoclast differentiation *in vitro* and *in vivo*	Mmp14, Mitf, Calcr, Traf6	Human breast cancer cell lines, intracardiac injected bone metastasis in mice	72
miR-33a	Inhibits lung cancer induced osteoclastogenesis	PTHrP	Human lung cancer cell lines *in vitro*	73
Let-7	Inhibits IL-6 in tumor-associated MSCs	IL-6	Human prostate cancer PC3 cells *in vitro*	74
Osteosarcoma				
miR-16	Tumor suppressor		Human osteosarcoma cells *in vivo* injected in mice	40
miR-126	Inhibits invasion, induces apoptosis	Sox2	Human osteosarcoma cells *in vitro*	41
miR-183	Inhibits migration/invasion,	Ezrin	Human osteosarcoma cells *in vitro*	42
miR-143	Inhibits migration/invasion, suppresses lung metastasis	MMP13	Human osteosarcoma cells inoculated in knee, intravenous injection of miR-143	43
miR-27a	Enhances migration, bone metastasis		Human osteosarcoma cells *in vivo* injected in mice	40
miR-21	Enhances migration/invasion	RECK	Human osteosarcoma cells *in vitro*	44
miR-140	Chemoresistance to methotrexate and 5-fluorouracil	HDAC4	Human osteosarcoma cells *in vitro*	46
miR-215	Chemoresistance to methotrexate and Tomudex	DHFR, TS	Human osteosarcoma cells *in vitro*	45

Abbreviations: IL-6, interleukin-6; MSC, mesenchymal stem cell.

tumor growth *in vivo*.[54] Conversely, miR-221 and miR-222 were frequently upregulated in MM patients, and expression of miRNA inhibitors to miR-221/222 decreased the proliferation and tumorigenesis of MM cells *in vitro* and *in vivo*.[55]

Tumor-Intrinsic MiRNA Regulation of Bone Metastasis

Bone metastasis, frequently observed in the advanced stages of breast and prostate cancers, represents the dysregulation of bone developmental pathways and a divergence from normal bone homeostasis. The presentation of metastatic lesions within the bone is responsible for pathological fractures, as well as for severe pain and hypercalcemia, representing a significant clinical concern.[56] Importantly, miRNAs can function in multiple aspects of bone metastasis regulation, including cell-autonomous mechanisms within tumor cells themselves, as well as through tumor–stromal interactions within the bone microenvironment (**Figure 1b** and **Table 2**).

The miRNA-mediated control of breast cancer bone metastasis

Tumor-intrinsic miRNA regulation has been shown to encompass nearly every aspect of breast cancer tumor progression. During metastasis, miRNAs can enhance or inhibit invasion, colonization to bone and secondary lesion outgrowth.[57] Let-7 has been shown to inhibit breast cancer invasion and bone metastasis in a mouse model, likely through the direct inhibition of *HMGA2*.[58] Similarly, miR-17 enhances

breast cancer cell line invasion and migration as well as metastasis.[59] When miR-17 expression was inhibited, Liu *et al.*[59] observed a decrease in proliferation and *in vitro* migration. A number of miRNAs have also been shown to negatively influence breast cancer migration and invasion. Ectopic expression of miR-335, which inhibits invasion through direct targeting of *Sox4* and *Tenascin C*, was shown to reduce breast cancer bone metastasis in an orthotopic xenograft model,[60] as well as in a model for small-cell lung cancer bone metastasis.[61] In addition, miR-206 expression significantly inhibited *in vitro* migration and invasion as well as tumor dissemination to the bone.[60]

In addition to regulating cellular motility, miRNAs have also been shown to influence tumor seeding and activity within the bone. MiR-218 stimulates the Wnt pathway in breast cancer cells by directly targeting the Wnt inhibitors *SOST*, *DKK2* and *SFRP2* that have the potential to enhance the expression of osteoblastic genes in metastatic cells.[62] This osteomimetic gene expression has the potential to influence metastatic cell homing to the bone. An additional study found that miR-204, miR-211 and miR-379 regulate interleukin (IL)-11 expression in breast cancer cells.[63] IL-11 has been previously shown to enhance osteolytic bone metastasis in breast cancer cells, and ectopic expression of these miRNAs in tumor cells might represent a novel therapeutic strategy.[64] Silencing of miR-126 in the human breast cancer MDA-MB-231 cell line was found to increase metastasis to multiple organs, including to bone.[65] MiR-126 expression inhibits endothelial cell recruitment to breast cancer cells, suppressing angiogenesis and distant colonization, through direct targeting of *IGFBP2*, *PITPNC1* and *MERTK*. Thus, inhibition of metastasis by miR-126 functions through a non-cell-autonomous mechanism resulting from decreased tumor–stromal interactions. Importantly, the signaling defects induced by miR-126 could be rescued by direct co-injection of tumor and endothelial cells.[65]

These studies underscore the therapeutic potential of miRNAs in inhibiting the molecular events preceding breast cancer bone metastasis. Interestingly, ectopic activation of miR-31 stimulates regression of established bone metastasis lesions, decreasing the number of existing lesions while preventing the outgrowth of residual metastatic nodules.[66] Although the exact mechanism behind this inhibition remains unclear, the authors observed a significant decrease in Akt activity after treatment with miR-31 that was tied to indirect inhibition through upstream targeting of ITGA5, RDX and RhoA.

Prostate cancer bone metastasis is mediated by miRNA regulation

Similar to breast cancer, late-stage prostate cancer patients frequently exhibit pathological complications related to bone metastasis. Consistent with findings from breast cancer, prostate cancer cells exhibit precise control of miRNA regulation. For example, miR-203 was found to function in an antimetastatic role in prostate cancer cell lines. Saini *et al.*[67] found direct targeting of a number of important genes for metastasis progression, including *Zeb2*, *Bmi*, *Survivn* and *Runx2*. Importantly, reintroduction of miR-203, which was repressed in metastatic cell lines relative to normal prostate cells, attenuated bone metastasis in a mouse model. In addition, miR-16 was shown to inhibit bone metastasis in a mouse prostate cancer xenograft model.[68] MiR-146a was shown to target *ROCK1* in androgen-independent PC3 cells; overexpression of miR-146a led to reduced proliferation and invasion, and impaired adhesion to a monolayer of human bone marrow endothelial cells.[69]

Finally, multiple studies have shown inhibitory effects from miR-145 on prostate cancer bone metastasis. Guo *et al.*[70] found that miR-145 can directly inhibit *HEF1*, leading to decreased migration and invasion, and decreased bone seeding of human prostate cancer cell lines. A separate study found that both miR-143 and miR-145 decreased in bone metastatic samples compared with primary tumors, and ectopic expression reduced migration, invasion and bone metastasis.[71]

MiRNA Regulation of Tumor–Stromal Interactions during Bone Metastasis

In addition to tumor-intrinsic effects, miRNAs have recently been shown to influence the essential tumor–stromal interactions that mediate bone metastasis. During osteolytic metastasis, tumor cells co-opt the regulatory signaling within bone marrow cells to recruit and activate osteoclasts that in turn orchestrate bone degradation. Recently, a number of miRNAs have been shown to directly influence osteoclast activity during bone metastasis. We found that a number of miRNAs are repressed during osteoclast differentiation, including miR-33a, miR-133a, miR-141, miR-190 and miR-219.[72] Interestingly, *in vitro* osteoclastogenesis assays revealed a necessity for this miRNA downregulation, with ectopic expression inhibiting osteoclast differentiation. Systemic inoculation of pre-miRNA oligonucleotides for miR-141 or miR-219 was sufficient to inhibit bone metastasis from human breast cancer cells. At the same time, miR-16 and miR-378, which were upregulated in patient serum samples with bone metastasis, possessed comparable sensitivity relative to the bone turnover marker N-terminal telopeptide (NTX).[72] A similar study found that miR-33a could target *PTHrP*, a known activator of osteolytic bone resorption, decreasing the ability of lung cancer cells to induce osteoclast differentiation and activity.[73] Thus, it is possible for miRNAs to inhibit bone metastasis through perturbation of tumor–stromal interactions.

Stromal cell-derived miRNAs are also capable of directly influencing metastatic cells. Let-7c inhibits IL-6 within bone marrow mesenchymal stem cells (MSCs), and ectopic expression of Let-7c was capable of repressing the migration- and invasion-promoting potential of tumor-associated MSCs.[74] A separate study found that multiple miRNAs were transferred from bone marrow stromal cells to nearby breast cancer cells upon seeding to the bone.[75] MiR-127, miR-197, miR-222 and miR-223, all direct inhibitors of *CXCL12*, were shown to directly transfer from stromal cells into cancer cells via gap junctions or through secreted exosomes. The transfer of these miRNAs decreased cancer cell proliferation and could represent a mechanism for tumor quiescence within the bone microenvironment.

Exosome-Derived MiRNAs during Bone Metastasis

In addition to the biological significance of intracellular miRNAs, recent studies have revealed the functional importance of secreted miRNAs. MiRNAs can be released from cancer cells within microvesicles or exosomes that are subsequently taken

up by distant cells.[76] Although the examination of exosome-delivered miRNAs has been shown in multiple cells, including microvesicles derived from dendritic[77] and T cells,[78] the process has not been comprehensively studied within bone metastasis. Ectopic expression of miR-192 in bone metastatic lung cancer cells resulted in secretion of the miRNA within microvesicles that could be transferred to endothelial cells *in vitro*.[79] Importantly, the transfer of miR-192 to the endothelial cells reduced angiogenic activity *in vitro*, and systemic injection decreased osteolytic lesions in a mouse model of bone metastasis.[79]

Intravenously injected exosomes are detectable in the bone marrow of mice;[80] thus, the potential exists for exosome-delivered miRNAs to serve as a therapeutic strategy for bone-related pathologies. Further research is required to monitor the efficiency of targeting to specific cells, both within the bone marrow microenvironment and tumor of origin, and to minimize off-target effects. In addition to therapeutic delivery, the detection of circulating miRNAs within exosomes holds the potential to serve as biomarkers for disease, with specific circulating miRNA signatures potentially serving as an indication of disease progression.[81]

Conclusions

The miRNA activity represents an essential level of gene regulation during skeletal development and homeostasis. Unbiased ablation of miRNA activity, through the inhibition of miRNA processing pathway components such as Dicer and Drosha, has been shown to inhibit the development of multiple bone marrow stromal cells, resulting in devastating skeletal defects. Misregulation of individual miRNAs is capable of inhibiting the differentiation and activity of multiple cell types and precipitating pathological events. Importantly, miRNA regulation has also been shown to play pivotal roles in regulating the development and progression of osteosarcoma, multiple myeloma and bone metastasis. Thus, proper miRNA expression is essential for bone formation and maintenance.

From a clinical standpoint, miRNAs involved in bone disease, osteosarcoma and bone metastasis might serve as an attractive therapeutic target. However, the development of miRNA-based therapeutics will require a more complete understanding of miRNA–mRNA targeting in diverse tissue types in order to minimize the potential side effects due to the multi-target nature of miRNAs in gene regulation. Nevertheless, preliminary studies have shown promising results in inhibiting osteosarcoma and bone metastasis, suggesting the potential utility of this therapeutic strategy in clinical practice. Taken together, the therapeutic potential of miRNA treatments has the ability to transform clinical paradigms for bone-related diseases.

Conflict of Interest

The authors declare no conflict of interest.

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