MicroRNAs in the control of metastatic bone disease

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Abstract

Bone metastasis is a common and devastating complication of late stage breast and prostate cancer. Complex interactions between tumor cells, bone cells and a milieu of components in their microenvironment contribute to the osteolytic, osteoblastic or mixed lesions present in patients with metastasis to bone. In the last decade, miRNAs have emerged as key players in cancer progression yet the importance of miRNAs in regulating cancer metastasis to bone is now being appreciated. Here, we emphasize important concepts of bone biology and miRNAs in the context of breast and prostate cancer and focus on recent advances that have improved our understanding of the role of specific miRNAs with direct involvement in metastatic bone disease.

Keywords

Breast cancer; Prostate cancer; miRNA; Bone metastasis; Osteolytic /Osteoblastic disease

Introduction

Cancer progression is classically described as a multistep process in which malignant cells survive an arduous journey of proliferation at the site of origin, invasion, intravasation, circulation, extravasation and growth at distal sites. The concept of cancer dissemination to distal sites has been recognized for more than a century, initially due to Steven Paget's seed and soil hypothesis, derived from his observations in patients revealing a propensity for breast cancers to spread to specific secondary sites [1]. Breast and prostate cancer are two of the most prevalent cancers worldwide and 65–75% of patients will suffer from bone metastases and skeletal related events, severely increasing both the morbidity and mortality rates of the disease [2]. To date, research has continued to unravel the intricacies of breast and prostate cancer metastases to bone; however, this remains the most clinically relevant yet poorly understood aspect of the disease. Metastatic bone diseases arise as a result of perturbed bone remodeling due to complex interactions between cancer cells and the bone microenvironment. Current research efforts are now addressing these interactions by

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investigating tumor cell responses in the bone, intriguingly both before and after engaging with the milieu of components present in the metastatic bone microenvironment (reviewed in [3]).

MicroRNAs (miRNAs) are emerging as important mediators of epigenetic control in normal and pathological cellular activities in distinct tissue environments. These small, 21–26 nucleotide-long single stranded RNA molecules have materialized as crucial post-transcriptional regulators of a variety of biological processes including skeletal homeostasis and pathology. In the last decade, countless studies have revealed miRNAs intimately involved in the initiation and progression of cancer (for recent reviews see [4, 5]). In this review, there will be a specific focus on miRNAs expressed in breast and prostate cancer cells that are often dysregulated in the primary tumor and importantly, coordinate a multitude of processes leading to bone metastasis. Indeed, a number of miRNAs have recently been described that regulate the normal homeostatic activity of different cell types in the bone required for bone formation (e.g. mesenchymal osteogenic lineage cells) and remodeling/turnover (e.g. hematopoietic derived osteoclasts)[4, 6, 7]. Moreover, some important components of bone biology will be considered to provide insight into how miRNAs in the bone microenvironment can contribute to metastatic phenotypes. Finally, we will briefly discuss recent developments in the therapeutic benefit of miRNAs in treating and/or preventing breast and prostate metastasis to bone.

Bone: a safe haven for tumor cells

To better understand how miRNAs influence the process of metastasis to bone, it is important to first briefly consider some of the major components of the tumor cell microenvironments at the primary site and importantly, in bone itself. The disturbance of tissue architecture at primary tumor sites that occur between the stromal support layer and the epithelial barrier of the gland is a consequence of cellular changes leading to the well characterized epithelial to mesenchymal transition (EMT), thought to be an initiating step in tumorigenesis [8]. The reverse phenomenon, denoted mesenchymal to epithelial transition (MET), has been identified in disseminated tumor cells in the bone marrow, further demonstrating the complex and dynamic nature of the bone microenvironment [9]. Both EMT/MET are now accepted as being regulated, at least in part, by miRNAs [10].

Bone is a complex tissue composed of multiple cell types including both bone forming and bone resorbing cells. Among the bone forming cells are subpopulations derived from mesenchymal osteoprogenitors. These bone-forming osteoblasts produce a mosaic of bone matrices, including woven bone, osteoid and mature bone. As osteoblasts terminally differentiate they become mineral-encapsulated osteocytes which function as responders to mechanical forces and physiological signals [11]. Osteoclasts are bone-resorbing cells, derived from the monocyte-macrophage lineage, that are crucial for the support and maintenance of mineral homeostasis and bone repair. Osteoblast and osteoclast cells operate in a tightly regulated process of bone formation and resorption, respectively. The critical signaling between these cells that supports their activities in response to hormonal stimuli (including VitD1,25(OH)2D3, parathyroid hormone and parathyroid hormone related

protein (PTHrP)) is primarily regulated through a RANK-RANKL-OPG axis. The aforementioned processes have been recently reviewed in [12].

Patients with metastasis to bone often present with different lesions that can be osteolytic, osteoblastic or a mixture of the two. Breast cancer bone metastases are mostly osteolytic, characterized by bone degradation as a result of increased osteoclast activity [13]. Conversely, prostate cancer bone metastases are predominantly osteoblastic (osteosclerotic), characterized by excessive bone formation resulting from augmented osteoblast activity (Figure 1) [14]. Once metastasizing breast and prostate cancer cells reach the bone, the type of bone lesion produced is based on the phenotype of the cancer cells and their interactions with various cellular and molecular components present in the bone microenvironment. This process was recently reviewed highlighting many of the molecules and signaling pathways involved including PTHrP; RANKL; OPG; IGF-1;TGFβ; IL-11; IL-6; OPN; BSP; M-CSF and others [2, 15]. Selected common mediators of bone metastatic disease in the context of breast and prostate cancer are highlighted below.

Transforming growth factor beta (TGFβ) is one of the most abundant growth factors in bone matrix and an important mediator of bone resorption and formation, as well as breast and prostate cancer and associated bone metastases [16]. In breast cancer, TGFβ released from the matrix as a result of increased bone resorption, can act on tumor cells to produce factors such as PTHrP and interleukin-11 (IL-11) that can perturb the RANKL/OPG balance, resulting in further osteoclastogenesis and perpetuation of osteolytic disease [17]. A recent study found that TGFβ stimulated Jagged1 and Notch signaling pathways resulting in IL-6 production, which promotes tumor cell proliferation in bone metastasis [18]. TGFβ has also been shown to interact with other environmental factors such as hypoxia in the bone microenvironment through stabilization of hypoxia inducible factor 1α (HIF1α) and enhanced induction of vascular endothelial growth factor (VEGF) and C-X-C chemokine receptor 4 (CXCR4), enhancing the metastatic ability of cells in the bone microenvironment [19].

PTHrP is thought to be one of the most important mediators of osteoclast activation and is produced by osteoblasts, stromal cells, and cancer cells. Many studies have demonstrated the importance of PTHrP in breast cancer progression and bone metastasis, with a reported 90% of bone metastases expressing PTHrP [20, 21]. Additionally, PTHrP expression has been shown to be an independent risk factor for predicting breast cancer bone metastasis [22]. PTHrP can accelerate osteoclastic bone resorption through increasing RANKL production in osteoblasts and consequently releases growth factors from the bone matrix including TGFβ and IGFs important for tumor growth and continued PTHrP secretion, contributing to the "vicious cycle" [23]. PTHrP has also been implicated in prostate cancer progression and bone metastasis and the mixed osteolytic and osteoblastic disease often present in patients with prostate cancer bone metastasis can be attributed to the functional activities of PTHrP [24].

Runx2 is a transcription factor known for its crucial roles in bone development, and its wide-ranging functions in breast and prostate cancer [25, 26]. Runx2 can modulate a variety of factors and signaling pathways (Wnt, BMPs, PTHrP, MMPs, TGFβ), favoring bone

metastasis [26]. More recent studies have revealed that hypoxia can up-regulate Runx2 expression, consequently activating anti-apoptotic factors such as B-cell lymphoma 2 (Bcl-2) in prostate cancer cells and that Runx2 can inhibit p53-dependent apoptosis in bone cells [27, 28]. Moreover, Runx2 was found to be a downstream mediator of the oncogenic PI3K/Akt pathway in breast cancer cells [29]. These findings suggest an important role for Runx2 in cancer cell survival, further substantiating the contribution of Runx2 to metastasis-related events in breast and prostate cancer.

The bone and bone marrow microenvironments harbor a myriad of additional cellular (e.g. stem, nerve and immune cells) and associated molecular components (e.g. SDF1/CXCR4, OPN, Wnt signaling, notch/jagged signaling), which make it a hospitable environment for cancer cells, with important implications for bone metastasis (recently reviewed by [30–33]). Although comprehensive review of the literature on this topic is outside of the scope of this review, some recent studies relevant to breast and prostate cancer are included. Shiozawa *et al* showed that the hematopoietic stem cell niche serves as a direct target for prostate cancer dissemination [34]. Moreover, using prostate cancer mouse models it was shown that hematopoietic progenitor cells could induce stromal cells to an osteoblastic phenotype and that hematopoietic stem cells could differentiate into osteoclasts via an IL-6 mediated pathway [35]. These studies reveal the central role hematopoietic stem/progenitor cells play in prostate cancer bone metastasis. Myeloid derived suppressor cells (MDSCs) are immature myeloid cells whose accumulation has been shown to suppress both innate and adaptive immune responses [36]. Recent studies have shown that these cells can function as osteoclast progenitors, resulting in enhanced bone loss in breast cancer [37]. Furthermore, Zhang *et al* revealed that specific types of breast tumors with a stroma rich in mesenchymal cytokines (CXCL12 and IGF1) are primed for adapting and flourishing in the bone metastatic environment [38]. Collectively, these studies offer new mechanistic insights into the dynamic nature of metastasis to bone.

Development and evolution of sophisticated genetic profiling techniques in recent years has significantly improved our knowledge and understanding of cancer progression. For example, almost a decade ago a model was proposed which pinpointed specific genes associated with colonization of cancer cells in bone [39]. studies have shown that specific microRNAs (miRNAs) are implicated in each step of cancer progression which are leading to profound changes in our understanding of the genetic control mechanisms that operate in all aspects of cancer, including metastasis [40]. The remainder of this review will focus on recent evidence highlighting specific microRNAs and their roles in breast and prostate cancer associated metastatic bone disease, as well as the potential opportunities for novel therapeutic interventions that this knowledge inevitably gives rise to.

miRNA actions

The miRNAs are one class of non-coding RNAs present in the genome that have the capacity to regulate genes with similar properties that can be linked to one or more specific pathways. They also are capable of controlling aberrant biological activities characteristic of tumor cells. It has been recently well recognized that miRNAs can be secreted from cells and delivered to recipient cells through a microvesicle/exosome-dependent mechanism, or

RNA-binding protein-associated active trafficking system. The biogenesis and selection of specific miRNAs in the synthesizing cell as well as the uptake and function in the recipient cell are still under intensive investigation, however it has been proposed that secreted miRNAs can function as endogenous miRNAs, regulating multiple target genes or signaling pathways [41]. MiRNA secretion and its role in intercellular communication has recently been considered [42] and thus will not be expanded upon in this review. However, it is important to recognize the possible novel role of miRNAs in cell-cell communication in the tumor and bone environment, as well as the already established clinical potential of using secreted miRNAs as disease biomarkers.

miRNAs and the pathogenesis of cancer

MiRNAs have been associated with the pathogenesis of an increasing number of cancers, including breast and prostate. Indeed, aberrant miRNA expression has been linked to both progression and clinical outcome of disease [43, 44]. Utilizing conventional microRNA profiling arrays, numerous groups have characterized common miRNAs that are dysregulated in solid cancers [45], miRNAs specific for tumor subtypes [46], as well miRNAs associated with metastasis [47] and disease recurrence [48].

High throughput analysis of the bone marrow of breast cancer patients with and without disease recurrence, followed by validation of a subset of these miRNAs, identified miR-21 and miR-181a as promising prognostic markers for breast cancer recurrence and survival [49]. In a similar study, Selth and colleagues found three miRNAs – miR-141, miR-146b-3p and miR-194 – to be overexpressed in prostate cancer patients who subsequently experienced biochemical disease recurrence [48]. The miR-106b-25 cluster of miRNAs has also been reported to be associated with prostate cancer disease recurrence, with over-expression of miR-106b in primary tumors being an independent predictor of early disease recurrence [50]. Additionally, using extensive datasets and deep sequencing approaches, miRNAs regulating the transition from *in situ* to invasive ductal carcinoma were discovered [51]. Development of *in situ* hybridization (ISH) techniques has facilitated validation of expression profiles obtained from high throughput studies in heterogenous solid tumor tissue [52]. Combining ISH with conventional immunohistochemistry allows simultaneous detection of miRNAs and their target protein expression in the same tissue section for co-localization and functional studies.

In addition to profiling miRNAs in tumor tissues, circulating miRNAs (c-miRNAs) have received intense attention. These c-miRNAs are considered stable in blood serum and plasma, and can be secreted in a variety of ways including from exosomes; as lipid particles bound to proteins; or as free miRNAs [53]. Successful characterization of c-miRNAs from breast and prostate cancer has revealed numerous miRNAs that are changed in disease sub-types, in response to chemotherapy as well as in bone metastasis [54–56]. Whether the c-miRNAs actually exhibit a functional role (as suggested by many investigators) is a compelling question that needs to be addressed in future studies.

miRNAs driving bone metastasis

The first miRNA shown to have functional relevance in metastasis was identified in 2007 [57]. miR-10b, regulated by Twist, is elevated in the serum of breast cancer patients with bone metastasis and in breast cancer tissue, where the expression correlates with disease progression and poor prognosis [54]. Functionally, miR-10b initiates and supports metastasis by direct targeting of homeobox D10, which in turn activates Ras homolog gene family, member C (RHoC) signaling, further aggravating the metastatic process. Since their initial discovery, a number of miRNAs are implicated in regulating various stages of the metastatic cascade in breast and prostate cancer metastasis to distal organs [58] (Table 1, Figure 1). Coordinate repression of metastasis-promoting genes (including integrin alpha5, radixin and RhoA) by miR-31 not only inhibits breast cancer metastasis but also causes disease regression of established metastasis in pre-clinical models [59, 60]. Besides being upregulated and targeted by miRNAs, Rho pathway associated kinase signaling has also been shown to up-regulate the metastasis-promoting miR-miR-17-92 cluster. Consequently, inhibition of either the Rho-associated protein kinase (ROCK) pathway or miR-17-92 diminished metastatic progression to bone and lung [61]. Recently, Png *et al* identified non-cell autonomous regulation of metastasis by miR-126 through targeting of the secreted pro-angiogenic and metastatic genes insulin-like growth factor binding protein 2 (IGFBP2), phosphatidylinositol transfer protein, cytoplasmic 1 (PITPNC1) and c-mer proto-oncogene tyrosine kinase (MERTK) [62]. Coordinate inhibition of these molecules in breast cancer cells results in impaired recruitment of endothelial cells, metastatic angiogenesis and colonization, emphasizing the potential of miRNAs to simultaneously regulate multiple pathways in a non-cell autonomous fashion.

Despite the extensive investigation on miRNAs regulating early events of breast and prostate cancer metastases and colonization to distant organs, the role of miRNAs in organ specific metastasis to bone is not well understood. A compelling question for targeted approaches to treatment and/or prevention of a metastatic event is to ask: what are the miRNAs driving metastases? Several studies have discovered miRNAs important in normal osteoblasts that promote bone formation [63, 64]. It was demonstrated that enhanced Wnt signaling effectively increased osteoblast production of the bone matrix proteins that also mediate homing of cancer cells to bone. Moreover, it was revealed that miR-218 promotes osteomimicry through stimulation of the Wnt pathway in bone-homing metastatic breast cancer cells [63]. Pollari *et al* recently identified a panel of miRNAs that are differentially expressed between parental MDA-MB-231 breast cancer cells and a bone metastatic MDA-MB-231 variant: miR-204, miR-211 and miR-379 were identified as negative regulators of IL-11, thus potentially contributing to the osteolytic bone destruction of breast cancer bone metastasis [65]. Examination of the miRNA expression profiles in primary and bone metastatic prostate cancer samples identified miR-143 and miR-145 expression to be downregulated in bone metastasis [66]. Consistently, overexpression of these miRNAs reduced migration and invasion *in vitro* and tumor progression and bone invasion *in vivo*, thus establishing miR-143 and miR-145 as suppressors of bone metastasis in prostate cancer. Follow up studies revealed the underlying mechanism of miR-145-mediated suppression of EMT through targeting of the oncogenic human enhancer of filamentation-1 (HEF-1) [67].

Recently, the first miRNAs directly associated with cancer-induced osteolytic bone disease were discovered by Ell *et al* [68]. Five miRNAs were significantly downregulated in osteoclasts during cancer-stimulated osteoclast differentiation and reconstitution of two of them, miR-141 and miR-219, impaired osteoclast function and prevented bone metastatic resorption. This study also reported miR-16 and miR-378 to positively correlate with bone metastatic burden in both mice and human patients, suggesting that these miRNAs can be used as biomarkers for metastatic bone disease.

Distinct mechanisms have been associated with regulation and deregulation of miRNAs. The selective deregulation of miRNAs in malignancy is often due to deletion, amplification or mutation of miRNA genes [69]. For example, the miR-15-miR-16 cluster is located in a genetically unstable locus in chromosome 13q14 and is frequently deleted in multiple cancers including prostate cancer [70]. These two miRNAs interfere with multiple oncogenic activities including Wnt signaling and cell cycle control thus serving as tumor suppressors in prostate cancer [71]. Besides inhibiting prostate tumor growth and contributing to the cross talk between tumor cells and their microenvironment through cancer-associated fibroblasts [72], miR-16 inhibits growth of metastatic prostate tumors. Takeshita *et al* demonstrated significant inhibition of tumor growth in bone by systemic delivery of miR-16 with atelocollagen, thus providing evidence for using miRNAs as therapy to prevent metastatic bone disease [73]. Additionally, miR-335 is located in chromosome locus 7q32.2 and often undergoes a genetic deletion and epigenetic promoter hypermethylation in metastatic patients. Indeed, miR-335 is implicated as a functional suppressor of tumor initiation and metastasis [62, 74].

As well as direct deregulation of miRNAs themselves, aberrant miRNA expression can be a result of genetic alterations of transcription factors and or epigenetic modulators acting on miRNA-encoding genes. Pandolfi's group elegantly showed miR-22-mediated epigenetic silencing of the anti-metastatic miR-200 and a subsequent increase in the stem cell compartment as well as enhancement of breast cancer development and metastasis [75]. The miR-200 family (which includes miR-200a, miR-200b, miR-200c, miR-141 and miR-429) is strongly implicated as an inhibitor of EMT, cell migration and breast cancer dissemination through direct targeting of Zinc finger E-box-binding homeobox 1 and 2 (ZEB1 and ZEB2) [76–78]. The miR-200 family has also been shown to promote metastatic colonization at distal sites by altering the tumor secretome through direct targeting of Sec23 homolog A (also known as SEC23A), involved in protein trafficking [79]. Another interesting concept on miRNAs regulating tumor-stroma interaction has been introduced by Rameshwar's group: they proposed that miRNAs can be transmitted from stromal cells to cancer cells in exosomes or via gap junctions, to regulate tumor cell dormancy [80]. The non-cell autonomous function of miRNAs is very likely to open new avenues of investigation and in the future it might reveal yet unknown mechanisms of miRNA function.

Concluding Remarks and Future Perspectives

An extensive knowledge base now exists for miRNAs involved in the regulation of tumor suppressors, oncogenic pathways and the metastatic event to distal sites. We are now challenged to further discover mechanisms contributing to deregulation of miRNAs in tumor

cells driving metastasis and importantly, translate these findings into clinical care. Another group of non-coding RNAs, which has recently emerged as being very relevant to breast and prostate cancer biology and metastasis is the long non coding RNAs (lncRNAs), such as HOTAIR and ANRIL [81–83]. Indeed, a very recent study showed that HOTAIR is regulated by miR-141 in select human cancer cell lines, adding another layer of complexity to the regulatory functions of miRNAs in cancer [84]. Given the impact of a decade or more of research on our understanding of the role of miRNAs in cancer, it will be interesting to see future research endeavors further unravel the importance of the lncRNAs in cancer and metastasis.

The future potential of miRNA-based therapy is in its infancy: although the effectiveness of specific miRNAs in metastatic bone disease is still under investigation, pre-clinical mouse studies have demonstrated promising results in targeting the metastatic tumor cells (as previously discussed), and the phase I clinical trial of miR-34 replacement therapy is currently underway [85].

One of fundamental questions that remain is whether the presence or absence of miRNAs is a cause or consequence of tumor cell activity in the primary tumor or at the metastatic site, especially in the unique environment of bone metastatic disease (see Outstanding Questions box). Addressing this issue requires an understanding of how miRNAs become dysregulated, which will be influenced by tumor cell factors that modify the biosynthesis of miRNAs. Additionally, signaling pathways upregulated in tumor cells have been identified that can promote their osteomimetic properties. For example, miRNAs that function in normal bone cells are aberrantly expressed in breast and/or prostate cancer, contributing to metastatic bone disease. Another concept that has not been fully explored is the fluctuation of miRNA expression in tumor cells that reside in the bone, as a result of their dynamic interactions in an ever-changing microenvironment.

Numerous publications have highlighted that miRNAs can exhibit pleiotropic and context dependent regulation of disease progression and site-specific metastasis. Indeed, miRNAs characteristic of a bone metastatic tumor can influence entire pathways supporting tumor growth and responses to the bone microenvironment. In addition, secreted miRNAs in bone can alter the normal activity of host cells to the advantage of tumor cells. Thus, the use of miRNAs as therapeutic agents is challenged by the need to achieve a delicate balance between ablating the tumor cells and rescuing normal cell activity. Taken together, these concepts emphasize the investigation of miRNAs specifically involved in bone metastasis to be not only compelling and complex, but crucial for working towards the goal of improved therapies for patients with metastatic bone disease.

Acknowledgments

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BOX 1

Outstanding Questions Box

- Are dysregulated miRNAs a cause or consequence of metastatic bone disease?

- How do circulating/secreted miRNAs function in mediating tumor interactions with the bone microenvironment?

- Can miRNA based therapeutic applications be developed for breast and prostate cancer metastasis to bone which meet the challenge of tumor cell specificity without further compromising homeostasis of the host environment?
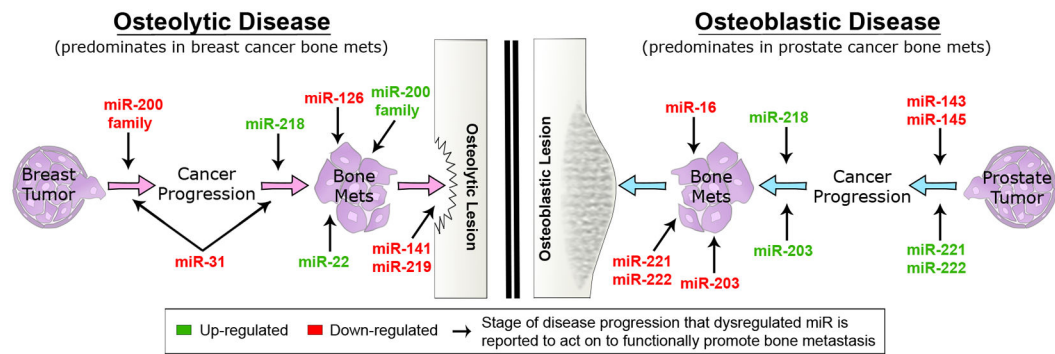
Figure 1. Dysregulated miRNAs in breast and prostate cancer driving metastasis to bone

Illustrated are osteolytic lesions (characterized by bone degradation) and osteoblastic lesions (characterized by excessive bone formation). Breast cancer bone metastases are osteolytic in nature, whereas osteoblastic lesions occur most commonly in prostate cancer bone metastases; indeed, often a mixture of both lesions is present. Specific miRs are highlighted in the illustration, based on their recently documented functional role(s) in promoting osteolytic or osteoblastic metastatic bone disease in breast or prostate cancer, respectively. If the miR is reported to be up-regulated/aberrantly present in the disease state, it is shown in green. Conversely, if the miR is reported to be down-regulated/aberrantly absent in the disease state, it is shown in red. Black arrows indicate the stage of disease progression. The stages are depicted from primary tumor formation through the metastasis stage and resultant bone lesion, where aberrant expression of the miRNA has been shown to functionally promote bone metastatic disease. We refer the reader to the main text ("miRNAs driving bone metastasis") as well as Table 1 for more detailed information on individual miRs depicted. Mets, metastasis; miR, microRNA.

Table 1

Selected miRNAs important in breast and prostate cancer disease progression

Selected miRNAs in this table have been included based on their involvement in breast and prostate cancer disease progression, with a focus on those that are linked with metastatic bone disease. The miRNA names, their reported role in breast and prostate cancer (function), reported gene targets, association with breast and/or prostate cancer, as well as their involvement in bone homeostasis are indicated.

miRNA	Function	Target	Tumor	Ref
miR-1	Inhibits cell proliferation and motility / Epigenetically silenced in prostate tumors and metastatic tissues	FN1, LASP1, PTMA, MCM7, CHK1, BRCA1, NOTCH3, γH2A.X	P	[86]
miR-10b	Required for initiation of breast cancer invasion and metastasis / Elevated in patients with bone metastatic breast cancer	HoxD10	B	[57]
miR-16	Inhibits tumor growth in bone / Downregulated in prostate cancer	CDK1, CDK2	P	[73]
miR-17-92	Positive regulator of migration, invasion and metastasis / Elevated in metastatic breast cancer cells	TGFbR2	B #	[61]
miR-21	Positive regulator of EMT, tumor cell growth, invasion and metastasis / Expression correlates with aggressive tumor and poor prognosis in breast and prostate cancer	PDCD4, MMP, MARCS, RHOB, tropomyosin 1 (TPM1)	B, P *	[87]
miR-23b/27b	Promotes breast cancer growth / Suppresses metastasis of breast cancer cells and castration-resistant prostate cancer cells / Low expression correlates with the development of metastases in breast cancer patients	Nischarin (NISCH) PAK2 kinase	B, P #	[88, 89]
miR-31	Inhibits invasion, extravasation and colonization / Expression regulated by promoter hypermetylation and inversely correlates with metastasis in patients	integrin-α5, radixin, RhoA	B #, *	[90]
miR-34a/c	Suppresses tumor growth regeneration of prostate cancer stem cells and metastasis / Epigenetically silenced in prostate cancer / Downregulation correlates with metastasis in breast cancer	CD44, c-myc, Notch4, Fra-1	B, P #	[87, 91]
miR-101	Inhibits breast and prostate cancer growth and invasion / Expression decreases during prostate cancer progression	EZH2, Stathmin1	B, P	[92]
miR-106b-25 cluster	Increases prostate cancer adhesion and growth / Associated with early recurrence in prostate cancer	Caspase 7	P	[50]
miR-125b	Tumor suppressor in breast cancer / Expression reduced in breast cancer	ETS1, ERBB2, ERBB3	B #	[44, 87]
miR-126	Inhibits metastatic colonization and endothelial recruitment by breast cancer cells / Silenced in human cancers	IGFBP2, PITPNC1, MERTK	B *	[62, 93]
miR-127, -197, -222, -223	Decrease breast cancer cell proliferation, activate quiescence / Regulate breast cancer through gap junctions and exosomes between bone marrow and breast cancer cells	CXCL12	B	[80]
miR-141	Inhibits osteolytic bone disease by attenuating osteoclast activity / Downregulated in osteoclasts by tumor cells	Mitf, Calcr	B *	[68]
miR-143, miR-145	Inhibit migration, invasion and metastasis	HEF1	B, P	[66, 67]

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miRNA	Function	Target	Tumor	Ref
miR-146	Expression decreased in bone metastatic biopsies compared to primary tumor Reduces angiogenesis, tumor growth, invasion and metastasis Downregulated in prostate cancer	ROCK1, EGFR, MMP2	P	[94, 95]
miR-155	Promotes migration and invasion, inhibits dissemination Biological function depends on the location of tumor cells Elevated in invasive breast cancer tissues and serum	RhoA, TCF4	B *	[87]
miR-196 family	Inhibits breast cancer migration and metastasis miR-196-Hoxc8 ratio correlates with metastasis	Hoxc8	B	[96]
miR-200 family	Inhibits EMT, migration and invasion of breast and prostate cancer Promotes metastatic colonization by regulating tumor secretome Silenced in breast and prostate primary tumor, elevated in metastatic tissue	ZEB1, SIP1, Sec23	B #	[76, 79]
miR-203	Inhibits EMT, invasion and motility Promotes MET in prostate cancer Expression attenuated in bone metastasis	ZEB2, Bmi, Survivin, Smad4	B, P	[97]
miR-204 miR-211 miR-379	Inhibit TGF-β-induced production of IL-11 Downregulated in bone seeking breast cancer cells	IL-11	B #	[65]
miR-205	Inhibits EMT and metastasis Underexpressed in breast and prostate tumors	ErbB3, VEGF, ZEB1, ZEB2, Protein kinase C, epsilon (PRKCE)	B, P #	[98]
miR-218	Promotes osteomimicry of bone metastatic breast cancer cells Overexpressed in metastatic breast cancer cells Overexpressed in high grade localized prostate cancer	Sost, TOB1	B, P #	[63, 95]
miR-219	Inhibits osteolytic bone disease by attenuating osteoclast activity Downregulated in osteoclasts in response to tumor	Traf6	B *	[68]
miR-221/221	Promote prostate cancer growth and EMT in breast cancer Highly expressed in prostate and breast cancer Downregulated in metastasis	p27(Kip1), ECTD2, RAB1A, TRPS1	P	[99, 100]
miR-335	Suppresses tumor initiation, migration, invasion and metastatic colonization Lost in tumors of patients who relapse, loss of expression predicts poor metastasis-free survival	Sox4, TenascinC	B #	[62, 93]
miR-373/ miR-520c	Promotes migration, invasion and metastasis in breast and prostate cancer Downregulated in prostate cancer Upregulated in breast cancer metastasis biopsies	CD44, RELA, TGFBRII	B, P	[87]

B, breast tumor; P, prostate tumor; Ref, reference;

reported in osteoblasts;

* reported in osteoclasts.