MicroRNA Exert Macro Effects on Cancer Bone Metastasis

Scott R. Baier 1 · Yihong Wan 1,2

Abstract Bone metastasis is a deadly complication of cancers arising from many different primary tumor locations. Cross talk between cancer and bone cells is a well-established driver of bone metastasis, and recent work reveals microRNA (miRNA) as key players in this communication. Functional significance of miRNA was first demonstrated in cancer cells and has now also been documented in bone cell differentiation and skeletal remodeling. Review of recent literature highlights how different miRNAs can impact each step of the metastatic process by acting in both tumor and the metastatic niche to exert pleiotropic effects. Additionally, whether a miRNA is ultimately pro- or anti-metastatic depends on the context—varied or even opposite outcomes can be conferred by the same miRNA in different cancer/cell types. In spite of this complexity, emerging research has provided a wealth of knowledge to uncover the exciting potential of miRNA as new diagnostic tools and therapeutic treatments for cancer bone metastasis.

Keywords MicroRNA · Cancer · Bone · Metastasis · Osteoclast · Osteoblast

Introduction

Due to advances in the treatment of primary tumors, overall cancer mortality has decreased approximately 22 % in the last 25 years [1]. In spite of the tremendous progress made, some tumors do not respond well to currently approved therapies and many of these eventually metastasize to bone tissue, including up to 70 % of advanced cases of breast and prostate cancer [2]. Because of the high incidence and mortality associated with bone metastasis, it is estimated that 350,000 people die each year in the USA with bone metastases [3]. In spite of the huge toll this places on patients and the health care system, many details about the regulation of the multi-step processes involved in bone metastasis remain unclear. Once bone metastasis has occurred, the prognosis is poor for most patients, as only 20 % of women with breast cancer survive 5 years after bone metastasis [4].

Bone metastasizing cancer cells take advantage of how bone tissue is in a constant state of remodeling. Typically, there is a tight coupling of bone formation by osteoblasts and bone resorption by osteoclasts. Osteoclasts originate from macrophage progenitors in the hematopoietic lineage in response to receptor activator of nuclear factor kappa-B ligand (RANKL) [5]. On the other hand, osteoblasts come from the mesenchymal lineage [6]. Either type of cell can become dysregulated during the metastasis of cancer cells leading to osteolytic or osteoblastic phenotypes [7]. Regardless of the type or if there is a mix of both, bone metastasis is associated with increased osteoclast activity and bone resorption [8, 9]. Moreover, recent studies provide evidence that tumor cells can also alter osteocytes—osteoblasts that become embedded in the bone matrix—to promote cancer bone metastasis [10, 11].

Once tumor cells arrive in bone tissue, a “vicious cycle” is initiated where bi-directional interactions between tumor cells and osteoclasts lead to bone loss and tumor growth. Tumor
cells produce factors such as RANKL and parathyroid hormone-related protein (PTHrP) that lead to increased osteoclastogenesis. With a greater osteoclast population in the microenvironment, there is increased bone resorption. To complete the cycle, the bone matrix releases more stimulatory growth factors such as transforming growth factor beta (TGFβ) and insulin-like growth factors (IGFs) that accelerate further tumor growth [12].

Recently, it has become apparent that miRNA also play a role in the development of bone metastasis. Reviews by White (2011), Browne (2014), and Ell (2014) highlight the importance of miRNA in bone cancer metastasis [13–15]. This review will build from these previous works by providing updated information since those reviews were published and will also detail findings on miRNA not discussed in those publications. Traditionally, miRNA have been viewed as intracellular regulators of biological function [16]. For a full review on miRNA synthesis and biology, please see the review by Krol and colleagues [17]. Briefly, following processing of premiRNA from enzymes in the nucleus and cytoplasm, mature miRNA (~21 nucleotides) act by binding to the 3′ UTR of target genes where they act by repressing translation, inducing target mRNA degradation, or via both functions [18]. The seed sequence of the miRNA, nucleotides 2–8, is key in mediating the regulatory effects due to structural complementarity to the target mRNA. Additionally, miRNA have demonstrated other activities including binding to other mRNA regions and binding to toll-like receptors [19, 20].

MicroRNAs Influencing Cancer Cell Proliferation, Epithelial-Mesenchymal Transition and Invasiveness

Dysregulation of miRNA can affect any part of the metastatic process. The first part of this review focuses on miRNA demonstrated to influence cancer bone metastasis primarily by inducing changes within cancer cells (Table 1). While it is well appreciated that miRNA regulate diverse biological processes, it has become increasingly evident in the recent years that tissue specificity is a key concept in miRNA biology. For instance, increased expression of miR-9 is generally regarded as being pro-metastatic as it positively regulates epithelial-mesenchymal transition (EMT), angiogenesis, and invasiveness [56–59]. However, those studies either focused on in vitro experiments or non-bone metastases. A comparison between small cell lung cancer cell lines found lower expression of miR-9 in SBC-5 cells that metastasize to skeletal and non-skeletal tissue as opposed to SBC-3 cells that only metastasize in non-skeletal tissue [41]. Mirroring the example of miR-9, recent findings have shown miR-409 can act in a similar fashion. In colorectal cancer, miR-409 has been demonstrated to protect against metastasis; but in the case of prostate cancer, it has been shown to activate the bone metastatic program [37, 60]. Another example of tumor-type specific effects can be observed with miR-96. Yu and colleagues have demonstrated a tumor-suppressor role for miR-96 in pancreatic cancers due to its ability to target KRAS [61]. On the other hand, a pro-metastatic role was recently described for miR-96 in prostate cancer [30]. Therefore, whether or not KRAS is the driving factor in tumor growth likely determines the role and importance of miR-96. Clearly, the location of the primary lesion and predicted site of metastasis are both important factors when considering the role of a given miRNA.

Overexpression of miR-10a and miR-10b is known to contribute to bone metastasis. Recent work has demonstrated that these miRNA genes are targets of Runx2, a known positive regulator of bone metastasis [21]. Additionally, miR-10b, known to play many roles in tumor progression, was recently shown to be a transcriptional target of TWIST1 [62, 63].

An emerging area of tumor miRNA biology is in the extracellular transfer of miRNAs via tumor-secreted vesicles. Exosomes are small, stable vesicles capable of mediating the transfer of miRNA from donor to recipient cells. The roles of cancer exosomal miRNAs in metastasis have been exemplified in two studies by the Wang group. In one study, breast cancer cell exosome-mediated transfer of miR-105 was shown to downregulate ZO-1 expression by targeting its 3′ UTR in vascular endothelial cells, ultimately promoting metastasis [33•]. In another study, breast cancer cell exosome-mediated secretion of miR-122 was shown to suppress glucose uptake in premetastatic niche cells by downregulating their glycolytic enzyme pyruvate kinase, thereby increasing cancer cell nutrient availability and facilitating metastasis [34].

A recent study also demonstrated the importance of the catalytic component of the RNA-induced silencing complex, argonaute 2 (AGO2), in cancer progression. This study showed miR-100 is able to target AGO2 and is downregulated in prostate cancer, a finding that aligned nicely with previous work showing overexpression of AGO2 enhances tumor metastasis [50]. Dicer, a key enzyme in miRNA processing, also can be a target of mature miRNA. Both miR-103 and miR-107 were shown to target Dicer in breast cancer cells, ultimately resulting in greater metastatic burden [31•].

MicroRNAs Affecting Cancer-Cell-Produced Mediators

Changes in the miRNA status of cancer cells can be the primary driver of tumor changes and progression, but cancer cells are not the only cells affected as aberrantly expressed miRNA have also been demonstrated to influence the bone microenvironment (Table 2). Taipaleenmäki and colleagues discovered a reciprocal relationship between the abundance of miR-135/203 and runt-related transcription factor 2 (Runx2) in metastatic breast cancer cells [68•]. In addition to
the known role of Runx2 in breast cancer progression, the authors showed miR-135 and miR-203 are normally abundant in breast epithelial cells but are dramatically reduced in metastatic breast cancer cells. By removing these miRNA from the cellular pool, inhibition of Runx2 is released and the metastatic program can advance. Ectopic expression of these two miRNAs decreased Runx2 abundance and reduced the secretion of Runx2 target proteins IL-11, MMP-13, and PTHrP, ultimately reducing metastatic disease progression [68]. Interestingly, the protective effects of miR-135/203 on metastatic progression may be further amplified when considering the effect of Runx2 on inducing pro-metastatic miRNA in cancer cells (see above) [21].

Targeting Runx2 expression is especially appealing because it is responsible for the induction of multiple genes involved in the metastatic pathway. However, in some cancers, individually targeting some of the downstream effectors of Runx2 may also decrease metastatic burden. Decreased

Table 1 MicroRNAs in cancer cells that influence bone metastasis by regulating proliferation, EMT, or invasiveness

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Cancer type</th>
<th>Known target(s)</th>
<th>Source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-10a/b</td>
<td>Breast</td>
<td>SMAD7, PDCD4, PTEN, SPRY2, COX-19</td>
<td>[22-27]</td>
</tr>
<tr>
<td>miR-21</td>
<td>Prostate, breast, lung</td>
<td>NOV/CCN3, TRAAD, CCNE1</td>
<td>[28, 29]</td>
</tr>
<tr>
<td>miR-96</td>
<td>Prostate</td>
<td>AKT1S1, FOXO1, FOXO3a</td>
<td>[30]</td>
</tr>
<tr>
<td>miR-103/107</td>
<td>Breast</td>
<td>Dicer</td>
<td>[31*, 32]</td>
</tr>
<tr>
<td>miR-105</td>
<td>Breast</td>
<td>ZO-1</td>
<td>[33]</td>
</tr>
<tr>
<td>miR-122</td>
<td>Breast</td>
<td>Pyruvate kinase</td>
<td>[34]</td>
</tr>
<tr>
<td>miR-124</td>
<td>Prostate</td>
<td>P4HA1</td>
<td>[35, 36]</td>
</tr>
<tr>
<td>miR-409*</td>
<td>Prostate</td>
<td>STAG2, RSU1</td>
<td>[37]</td>
</tr>
<tr>
<td>miR-541</td>
<td>Prostate</td>
<td>AR</td>
<td>[38]</td>
</tr>
<tr>
<td>miR-1</td>
<td>Prostate</td>
<td>TWIST1, SRC</td>
<td>[39, 40]</td>
</tr>
<tr>
<td>miR-9b</td>
<td>Lung</td>
<td></td>
<td>[41]</td>
</tr>
<tr>
<td>miR-15a/16</td>
<td>Prostate</td>
<td>BCL2, CCND1, CCNE1 and CDK4-6, Wnt signaling</td>
<td>[22]</td>
</tr>
<tr>
<td>miR-30a</td>
<td>Prostate, breast</td>
<td>ERG, MTDH</td>
<td>[42, 43]</td>
</tr>
<tr>
<td>miR-33a</td>
<td>Lung</td>
<td>HIF-1α, TWIST1</td>
<td>[44, 45]</td>
</tr>
<tr>
<td>miR-34a</td>
<td>Gastric, ovarian</td>
<td>AXL, MET, c-Myc, TCF7</td>
<td>[46-49]</td>
</tr>
<tr>
<td>miR-100</td>
<td>Prostate</td>
<td>AGO2</td>
<td>[50, 51]</td>
</tr>
<tr>
<td>miR-101</td>
<td>Lung</td>
<td>COX2</td>
<td>[52]</td>
</tr>
<tr>
<td>miR-125a/b</td>
<td>Breast</td>
<td>ERBB2, ERBB3</td>
<td>[53]</td>
</tr>
<tr>
<td>miR-335</td>
<td>Prostate</td>
<td>eNOS</td>
<td>[54]</td>
</tr>
<tr>
<td>miR-429</td>
<td>Breast</td>
<td>ZEB1, CRKL</td>
<td>[55]</td>
</tr>
<tr>
<td>miR-543</td>
<td>Prostate</td>
<td>eNOS</td>
<td>[54]</td>
</tr>
</tbody>
</table>

*a May suppress metastasis in other sites
*b May activate metastasis in other sites

Table 2 MicroRNAs that influence bone metastasis by targeting bone cells or secreted factors

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Effect on metastasis</th>
<th>Cancer type</th>
<th>Target(s)</th>
<th>Source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-33a</td>
<td>Suppresses</td>
<td>Lung</td>
<td>PTHrP</td>
<td>[64]</td>
</tr>
<tr>
<td>miR-34a</td>
<td>Suppresses</td>
<td>Breast, skin</td>
<td>TGF2</td>
<td>[65••]</td>
</tr>
<tr>
<td>miR-126</td>
<td>Suppresses</td>
<td>Prostate</td>
<td>VCAM1</td>
<td>[66••]</td>
</tr>
<tr>
<td>miR-130b</td>
<td>Suppresses</td>
<td>Prostate</td>
<td>MMP2</td>
<td>[67]</td>
</tr>
<tr>
<td>miR-135</td>
<td>Suppresses</td>
<td>Breast</td>
<td>RUNX2</td>
<td>[68•]</td>
</tr>
<tr>
<td>miR-141</td>
<td>Activates/suppresses</td>
<td>Prostate/breast</td>
<td>MITF/CALCR</td>
<td>[69, 70, 71••]</td>
</tr>
<tr>
<td>miR-203</td>
<td>Suppresses</td>
<td>Breast</td>
<td>RUNX2</td>
<td>[68•]</td>
</tr>
<tr>
<td>miR-219</td>
<td>Suppresses</td>
<td>Breast</td>
<td>CACR1</td>
<td>[71••]</td>
</tr>
<tr>
<td>miR-335</td>
<td>Suppresses</td>
<td>Lung</td>
<td>RANKL</td>
<td>[41]</td>
</tr>
</tbody>
</table>
expression of miR-33a in lung cancers results in a greater likelihood of bone metastasis through de-repression of PTHrP [64]. Similarly, loss of miR-130b in prostate cancer cells allows for greater MMP-2 expression, ultimately enhancing metastatic progression [67].

**MicroRNAs Involved in Bone Cells in the Skeletal Microenvironment**

Recent reports reveal that miRNA are also key regulators of bone metastatic niche cells and how they communicate with tumor cells (Table 2). Many cancers demonstrate a deletion of miR-34a, which helps drive the metastatic process [46–49]. In addition to the effects of losing miR-34a expression within the cancer cells, work from our laboratory has demonstrated the importance of miR-34a loss in osteoclasts during bone metastasis of cancers such as breast cancer and melanoma [65••]. Genetically, bone metastasis is exacerbated by osteoclastic miR-34a deletion but impeded by osteoclastic miR-34a over-expression. Pharmacologically, both bone resorption and bone metastasis are effectively attenuated by a miR-34a mimic systemically delivered via a nanoparticle vehicle. Mechanistically, miR-34a inhibits osteoclast differentiation by suppressing its targets including transforming growth factor beta-induced factor 2 (Tgif2), a pro-osteoclastogenic transcription factor. These findings suggest that miR-34a loss-of-function in osteoclasts may be a critical etiology of cancer bone metastasis, and that miR-34a replacement therapy can potentially limit bone metastasis from many types of cancers.

Important work by Tai and colleagues has provided an alternative example of how miRNA can become dysregulated during bone metastasis through interactions with bone cells. Typically, changes within cancer cells alone result in aberrant miRNA expression. However, in their study, osteoblast-secreted Wnt1-induced secreted protein-1 (WISP-1) led to decreased miR-126 expression in prostate cancer cells [66•]. Ultimately, this leads to a greater metastatic potential via increased vascular cell adhesion molecule-1 (VCAM-1) expression. Their results indicate that both WISP-1 inhibitors and miR-126 mimetics have potential utility as therapeutic agents for the prevention of prostate cancer metastasis to the bone.

Additionally, the effect of a given miRNA on the development of bone metastases is dependent on the type of cancer in question. Increased serum miR-141 has been strongly associated with metastatic prostate cancer [69, 70], yet miR-141

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**Fig. 1** A summary of recent findings on how miRNA regulate cancer bone metastasis
seems to offer a protective role against breast cancer metastasis [71••]. This dichotomy serves as further evidence that the context within which miRNA changes occur ultimately determine the total impact on bone metastasis.

Future Direction

The aforementioned publications have greatly increased our understanding in the recent years of the importance of miRNA in bone metastasis (summarized in Fig. 1). In spite of this new knowledge on the molecular pathways driving metastasis from the primary lesion to bone, more research is needed both to better understand which miRNAs best serve as molecular markers of metastatic disease and to advance the promising preclinical work of several groups to identify which miRNAs might effectively serve as therapeutics in the treatment or prevention of cancer bone metastasis. Furthermore, the development of specific and stable miRNA mimics and inhibitors, as well as efficient delivery vehicles such as nanoparticles and liposomes, will facilitate clinical application of these discoveries.

Differentiation of osteoclasts and osteoblasts is known to be dependent on the activity of miRNA [72]. Various studies have attempted to describe the changes in miRNA expression throughout the differentiation and maturation process of osteoclasts and osteoblasts, for examples see [15, 73–75]. Although it is outside the scope of this review to cover these miRNAs in detail, future functional studies are warranted to reveal whether and how these miRNAs play important regulatory roles in bone cell differentiation and cancer bone metastasis.

Conclusions

The emergence of microRNA as key players in cancer bone metastasis is clear. Many portions of the metastatic process can be influenced by aberrant miRNA transcription, and the importance of miRNA has been demonstrated in most cancers that commonly metastasize to bone. When designing future experiments in this area, researchers should be cautioned against blindly translating results from one cancer/cell type to another. Depending on the pool of available target miRNA in a cell, a particular miRNA may be pro-metastatic in one cancer and have no impact or even be protective in another. While the clinical use of circulating miRNA as biomarkers of metastatic disease or miRNA mimetics/inhibitors as cancer therapeutics are both promising strategies, much more work needs to be done for either to be applied on a wide scale in human patients. Problems with the delivery of therapeutics to bone and cancer cells while preventing off-target effects in other tissues are among the challenges issues to overcome.

A better understanding of both cancer and bone biology, however, is expected to facilitate these efforts.

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Compliance with Ethical Standards

Conflict of Interest Scott R. Baier and Yihong Wang declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human subjects performed by any of the authors. In a cited reference co-authored by Y. Wan [65••], all protocols for mouse experiments were approved by the Institutional Animal Care and Use Committee of University of Texas Southwestern Medical Center.

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Papers of particular interest, published recently, have been highlighted as:
• Of importance
  • Of major importance


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