Current Osteoporosis Reports (2019) 17:122–128
https://doi.org/10.1007/s11914-019-00510-4

CANCER-INDUCED MUSCULOSKELETAL DISEASES (E KELLER AND J STERLING, SECTION EDITORS)

MicroRNAs in Bone Metastasis

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Abstract

Purpose of Review This review provides an update on the recent literature describing the role of microRNAs (miRNAs) in cancer formation and bone metastasis. We confined our focus on osteosarcoma, breast cancer, prostate cancer, and epithelial-mesenchymal transition.

Recent Findings In all areas covered, major discoveries on the role of miRNAs in tumorigenesis and metastasis have been made. Novel signaling networks were identified with miRNAs having a central function. Potential improvements in the diagnosis of malignant diseases and the long-term follow-up might become possible by the use of miRNAs. Furthermore, miRNAs also have disease-modifying properties and might emerge as a new class of therapeutic molecules.

Summary MiRNAs are novel and important regulators of multiple cellular and molecular events. Due to their functions, miRNAs might become useful to improve the diagnosis, follow-up and treatment of cancer, and metastases. Thus, miRNAs are molecules of great interest in translational medicine.

Keywords microRNAs · Bone · Metastasis · Cancer · Biomarker · Therapy

Introduction

MicroRNAs (miRNAs) are highly conserved small single-stranded non-coding RNAs of about 18 to 26 nucleotides in length. By binding to a specific seed sequence in the 3′ untranslated region of a target messenger RNA (mRNA), miRNAs form anti-sense interactions with mRNAs and restrict the gene expression at the post-transcriptional level by reducing mRNA stability or by interfering with protein translation [1]. Since one miRNA can target several mRNAs, miRNAs are components of a larger regulatory network [2]. Thus, miRNAs are implicated in the regulation of multiple cellular functions in the context of development, differentiation, proliferation, apoptosis, and tissue homeostasis but also in cancer formation and metastases [3].

Deregulation of miRNA expression has been associated with several malignancies. It is well established that cancer cells secrete miRNAs into the circulation [4, 5]. Circulating miRNAs are stable in the blood stream and can therefore be used as biomarkers to support the diagnoses of a malignant disease or for long-term follow-up after therapy [6–9]. To date, more than hundred clinical trials are listed in *clinicaltrials.gov* aimed at investigating the usefulness of miRNAs as biomarkers in various diseases. It seems very promising that this approach could enter the clinics in the future [10, 11].

In the recent past, many insights have been obtained on the role of miRNAs in the pathophysiology of diseases. Signaling networks have been elucidated and potential diagnostic or therapeutic approaches have been exploited. Besides being useful for diagnostic purposes, miRNAs could also play a role in the treatment of diseases, including cancer [12, 13]. This article provides a brief update on the recent literature with the focus on the role of miRNAs in osteosarcoma, breast cancer, prostate cancer, and epithelial-mesenchymal transition.

Osteosarcoma

Osteosarcoma is the most frequent primary bone tumor with the highest prevalence during adolescence [14]. Detection of

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This article is part of the Topical Collection on *Cancer-induced Musculoskeletal Diseases*

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the tumor or its relapse is so far mainly based on imaging modalities. In the recent past, surgical techniques have been refined and a multi-agent chemotherapy became available that greatly improved the patient prognosis [15]. Despite these advancements, there is still a great need for further improving the care of patients with osteosarcoma. One of the problems in the clinics is the long-term monitoring for an early detection of recurrence or metastasis. However, there is no biomarker available that could be used in bone or soft tissue sarcoma. In this context, Fujiwara et al. contributed new knowledge by performing a global miRNA screen [16]. The group identified an osteosarcoma-associated serum-based miRNA signature that was further validated using in vitro and in vivo approaches. Their findings demonstrate that miR-25-3p was secreted by osteosarcoma cell lines and detected in tumor-derived exosomes [16]. Furthermore, osteosarcoma patients had high serum concentrations of miR-25-3p, which correlated with a poor prognosis [16]. Thus, this non-invasive monitoring approach could help to further improve the future clinical care of osteosarcoma patients.

A deregulated expression of the runt-related transcription factor Runx2 plays a critical role in the differentiation and proliferation of osteosarcoma cells [17]. Runx2 levels are also inversely linked to loss of p53, which is upstream of Runx2 and predisposes to osteosarcoma [17]. A study by van der Deen et al. revealed that the p53-dependent miR-34c is the most significantly downregulated Runx2-targeting microRNA in osteosarcoma [18]. The work further established a regulatory network by which p53 decreases Runx2 abundance in a miR-34c-dependent manner [18]. In a similar context, Zhang et al. described a negative correlation between miR-30a expression and the grade of malignancy of osteosarcoma cell lines [19]. The group further reported that miR-30a targets Runx2, thereby affecting the proliferation, migration, and invasion of osteosarcoma cells and eventually tumor formation and pulmonary metastasis in vivo [19]. These studies emphasize the importance of Runx2-targeting microRNAs in the context of osteosarcoma.

Two studies uncovered the importance of microRNAs regulating the abundance of breast cancer cell 2 (BRCC2) and its role in osteosarcoma. BRCC2 is a 12 kDa cytosolic protein containing a BH3-like domain at the N-terminus that causes a caspase-dependent apoptosis of cancer cells [20]. One study reported that miR-17-5p directly targets BRCC2, thereby promoting the growth of osteosarcoma [21]. The second study revealed that miR-603 also functions as an oncogene by suppressing BRCC2 protein translation in osteosarcoma [22].

Several other molecules and pathways are implicated in the pathophysiology of osteosarcoma and targeted by microRNAs. For instance, Liao and coworkers reported that the chemokine (C-C motif) ligand 3 (CCL3) promotes VEGF-A expression and angiogenesis in osteosarcoma by downregulating miR-374b expression via JNK, ERK, and p38 signaling [23]. Another study published by Xu et al. demonstrated that miR-146b-5p promotes proliferation, migration, and invasiveness of osteosarcoma cells [24]. Furthermore, miR-146b-5p increased the resistance to chemotherapy by decreasing the protein abundance of zinc and ring finger 3 (ZNRF3), suggesting a potential therapeutic implication [24]. FOS-Like antigen 2 (FOSL2) was shown by Sun and colleagues to be a direct target of miR-143-3p [25]. Expression of miR-143-3p was lower in osteosarcoma and in patients with poor prognosis. While proliferation and metastasis was inhibited by miR-143-3p, apoptosis was promoted. Consistently, FOSL2 expression was higher in osteosarcoma cells and its overexpression reversed the effects of miR-143-3p. Thus, miR-143-3p inhibits the proliferation, migration, and invasion in osteosarcoma by targeting FOSL2 [25]. The chemokine receptor 4 (CXCR4) is involved in tumor cell proliferation, migration, and invasion [26]. CXCR4 expression is increased in osteosarcoma, supports the formation of lung metastases, and is a novel target of miR-613 [27]. Thus, overexpression of miR-613 decreased osteosarcoma cell proliferation and induced apoptosis, thereby establishing a novel mir-613-CXCR4 regulatory signaling in osteosarcoma [27]. Using a comparative miRNA expression profile including osteosarcoma, circulating tumor cells and lung metastases from mouse xenograft models, Georges and co-workers uncovered that the expression of miR-198 and miR-206 was decreased in lung metastatic samples [28]. Confirmation of this finding in patient biopsies from matching primary tumors and lung metastasis suggested that miR-198 and miR-206 might have an anti-metastatic effect, which was indeed confirmed using in vitro assays. In vivo, intra-tumoral injections reduced tumor burden and prolonged the survival time of the treated animals. Thus, miR-198 and miR-206 might be of diagnostic or even therapeutic use in the treatment of osteosarcoma [28].

A very important aspect in cancer biology is the regulation of cancer stem cells. Lu et al. identified that miR-26a is downregulated in osteosarcoma stem cells [29••]. Consistent with this finding, miR-26a overexpression inhibits osteosarcoma cell growth in vitro and in vivo. In osteosarcoma patients, reduced miR-26a expression is associated with lung metastasis and a reduced overall survival. Mechanistically, miR-26a targets the Notch ligand Jagged 1 and its effect as tumor suppressor is mediated through inhibition of Jagged1/Notch signaling [29••]. These studies altogether support the notion that miRNAs might be of diagnostic and therapeutic value in the context of osteosarcoma.

Breast Cancer

Breast cancer is the most commonly occurring cancer in women worldwide. Most of the breast cancer-related deaths are due to metastatic spread into distant organs with bone being the most common site [30]. Approximately 70% of patients with metastatic breast cancer suffer from bone metastases. Bone metastases cause severe pain, pathological fractures, life-threatening hypercalcemia, spinal compression, and immobility and increase the mortality [31]. Although clinical management of metastatic bone disease, including surgical approaches, radiation therapy, and targeted drug therapy, has greatly improved, these treatment approaches are only palliative and the disease remains incurable. In addition, no clinically reliable biomarkers are available for the prediction and diagnosis of bone metastases [32]. Thus, the identification of deregulated miRNAs that control metastatic bone disease has been a major breakthrough and encouraged researchers to further investigate their role as potential biomarkers and therapeutic targets of breast cancer-induced metastatic bone disease [33].

To survive in the bone microenvironment, bone seeking metastatic breast cancer cells express several bone-related genes that are expressed by osteoblasts and osteoclasts under physiological conditions, including the master regulator of osteoblast differentiation Runx2 [34••]. This process is defined as osteomimicry and promotes metastatic tumor growth and osteolytic disease but can be attenuated by miRNA replacement strategies [34••, 35, 36]. Several miRNAs are predicted to target Runx2 [35]. Among them, the miR-30 family members, miR-135a and miR-203, were downregulated in bone metastatic MDA-MB-231 breast cancer cell lines compared to their parental counterparts or non-malignant breast epithelial cells [35]. Consistently, the expression of miR-135 and miR-203 was low in bone metastases obtained from breast cancer patients, while a low expression of miR-30 in primary breast tumors was associated with a poor relapse-free survival. Loss of the miR-30 family members, miR-135 and miR-203 in aggressive breast cancer cell lines and in patients indicated a tumor suppressor function of these miRNAs in bone metastatic breast cancer. Indeed, in a series of in vitro and in vivo experiments, Croset et al. recently demonstrated that overexpression of miR-30 in breast cancer cells inhibits cancer cell invasion, reduces tumor growth in bone, and attenuates bone destruction [36]. Besides Runx2, miR-30 was shown to directly target multiple bone metastases-associated genes including Cadherin 11 (CDH11) and Integrin alpha 5 (ITGA5), indicating that the miR-30 family alleviates the bone metastatic burden through multiple mechanisms. Pharmacological replacement of miR-135 and miR-203 in MDA-MB-231 breast cancer cells impaired the orthotopic tumor growth and spontaneous metastasis to bone in a Runx2-dependent manner [35]. Furthermore, delivery of miR-135 and miR-203 mimic oligonucleotides reduced the tumor growth in the bone marrow microenvironment and alleviated osteolytic disease [35].

In addition to transcription factors, bone-related signaling pathways, including the Wnt pathway are frequently activated in bone metastatic breast cancer cells [37]. Concomitant with active Wnt signaling, miR-218 expression was found to be increased in bone metastatic breast cancer cells and in bone metastases biopsies obtained from breast cancer patients [38, 39]. Furthermore, miR-218 was elevated in the serum of breast cancer patients with bone metastases compared to patients without bone involvement and secreted in extracellular vehicles by metastatic breast cancer cells [39]. Consistently, delivery of miR-218 in breast cancer cells promoted bone metastases progression and osteolytic disease in vivo while pharmacological inhibition of miR-218 alleviated the disease [38]. Mechanistically, miR-218 was shown to target several Wnt signaling inhibitors, including Sclerostin and sFRP-2 to promote Wnt signaling and the secretion of the Wnt target PTHrP, leading to an enhanced osteoclast differentiation and bone destruction [38]. Intriguingly, miR-218 secreted by cancer cells was taken up by osteoblasts in which it repressed type I collagen production, leading to a further imbalance between bone formation and bone resorption [39].

To induce osteolytic bone destruction, breast cancer cells secrete cytokines that directly or indirectly activate osteoclasts. Interleukin 11 (IL-11) has well-characterized functions as a hematopoietic growth factor and pro-oncogenic cytokine [40]. In addition, breast cancer-derived IL-11 sustains the pool of osteoclast progenitor cells, thereby augmenting osteoclastogenesis [41]. Recently, Cai et al. identified IL-11 as a direct target of a tumor-suppressor miRNA, miR-124 [42]. Beyond its role in the pathogenesis and metastasis of various tumors, miR-124 was revealed to inhibit breast cancer bone metastases and cancer-induced osteoclast activity. Further gain of function and loss of function studies demonstrated that breast cancer cell-derived miR-124 inhibits osteoclast survival and differentiation in an IL-11-dependent manner [42]. In patients, miR-124 was reduced in bone metastases compared to primary tumor tissue and low expression of miR-124 was associated with a shorter bone metastasis-free and overall survival. In contrast, high levels of IL-11 were associated with poor survival and inversely correlated with miR-124, highlighting the clinical relevance of the miR-124/IL-11 axis in breast cancer bone metastases [42].

Besides regulating osteoclast-stimulating cytokines in breast cancer cells, miRNAs directly act on osteoclasts in the metastatic bone environment [30]. For instance, Krzeszinski and colleagues used a number of genetic and pharmacological models to show that miR-34a-5p inhibits physiological and pathological bone resorption and attenuates bone metastases of breast and skin cancer [34••]. Transforming growth factor-β-induced factor 2 (Tgif2) was identified as a novel pro-osteoclastogenic factor and as a crucial target of miR-34a. In patients, miR-34a-5p expression was elevated in ductal breast carcinoma tissue compared to healthy mammary gland but almost absent in bone metastases [34••]. Collectively, these findings suggest that pharmacological replacement therapy of miR-34a might be an attractive therapeutic strategy to ameliorate breast cancer-induced bone disease.

Prostate Cancer

To date, prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer-related deaths in men [43]. Similar to breast cancer, the skeleton is a very common site for prostate cancer metastases, which greatly impair the quality of life and the overall survival time [44]. Thus, novel approaches are also needed for the better care of patients with prostate cancer and several studies reporting new findings on miRNAs have been published recently. For instance, Bonci et al. reported that loss of miR-15 and miR-16 in combination with an increased expression of miR-21 activated TGF-β and hedgehog signaling and promoted the spreading of prostate cancer cells, bone marrow colonization, and bone destruction [45]. Huang and coworkers determined that the expression of miR-141-3p was lower in bone metastatic prostate cancer tissues compared with non-bone metastatic prostate cancer tissues [46]. One of the proposed mechanisms was that an increased miR-141-3p expression suppressed the epithelial-mesenchymal transition (EMT), an important mechanism in cancer development and progression that is further discussed below. Furthermore, higher levels of miR-141-3p reduced the invasion and migration of prostate cancer cells while silencing of miR-141-3p had opposite effects [46]. Mechanistically, in addition to suppressing EMT, miR-141-3p targets the tumor necrosis factor receptor-associated factor 5 (TRAF5) and 6 (TRAF6), leading to an inhibition of NF-κB signaling and the attenuation of the metastatic features. These observations underscore the notion that miR-141-3p mimics might be of therapeutic use in the context of prostate cancer metastases [46]. The role of activated TGF-β signaling in promoting bone metastasis of prostate cancer was also emphasized in a recent study by Wa et al. [47]. The group reported that miR-19a-3p expression is decreased in bone metastatic prostate cancer cells and tissues [47]. Upregulation of miR-19a-3p reduced invasion, migration, and bone metastasis formation by targeting SMAD2 and SMAD4 and therefore inactivating TGF-β signaling [47]. These studies greatly contribute to the better understanding of the role of microRNAs in metastatic prostate cancer and might open avenues for future diagnostic or therapeutic approaches.

Epithelial-Mesenchymal Transition

During EMT, epithelial cells lose their cell polarity and cell-cell adhesion, causing a detachment of epithelial cells and the initiation of an altered transcriptional program [48]. EMT plays an important role in several biological processes including metastasis and resistance to therapeutic regimens [48]. Hence, preventing EMT as a form of cellular plasticity is a powerful approach to reduce cancer formation, metastatic spread, and resistance to therapy [49]. In osteosarcoma, Chen et al. described that miR-130a expression is significantly increased in osteosarcoma and related with the formation of metastases and a compromised prognosis of osteosarcoma patients [50]. Using functional studies, the team uncovered that miR-130a enhanced EMT and therefore the migration and invasion of osteosarcoma cells [50]. This augmented metastatic properties appeared to be mediated by targeting the phosphatase and tensin homolog (PTEN) [50].

Chondrosarcoma is another primary tumor of the musculoskeletal system that is rather resistant to radiotherapy and chemotherapy [51]. Furthermore, chondrosarcoma has a high rate of death in children and adolescence, indicating the great need for improved therapies [51]. Wu et al. identified that in chondrosarcoma, miR-300 targets Twist, leading to an inhibition of EMT [52]. Furthermore, endothelin-1 (ET-1), a factor known to promote chondrosarcoma angiogenesis and metastatic spread [53], promotes EMT in

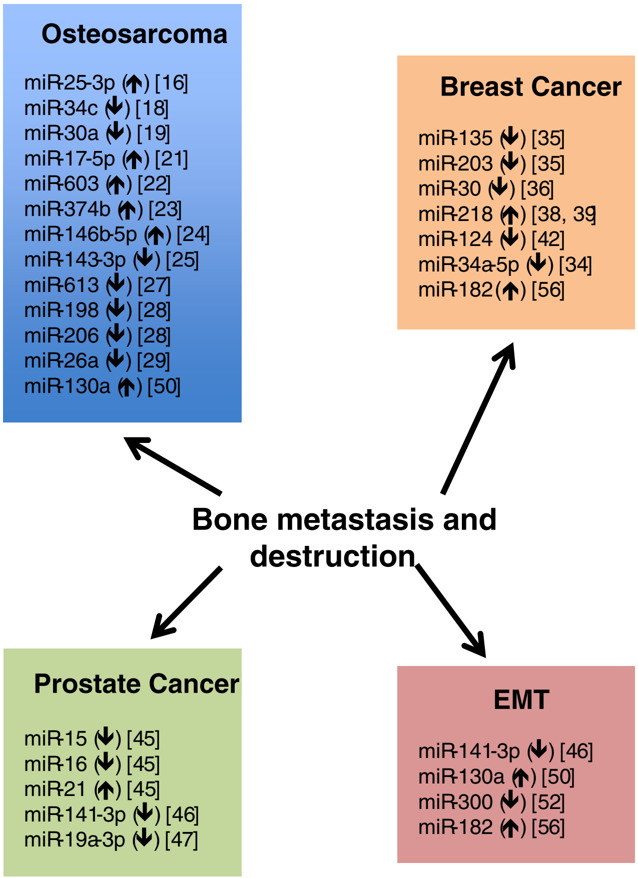

Fig. 1 Overview of microRNAs (miRNAs) participating in bone metastasis and destruction. Shown are miRNAs implicated in the development and progression of osteosarcoma, breast cancer, prostate cancer, and epithelial-mesenchymal transition (EMT), whether the miRNA is upregulated or downregulated and the related reference cited in this article

human chondrosarcoma cells [52]. ET-1-mediated induction of EMT occurs through the AMP-activated protein kinase (AMPK) pathway and repression of miR-300 [53]. Thus an ET-1-AMPK-miR-300-Twist signaling cascade regulates EMT in chondrosarcoma cells.

TGF-β signals through SMAD-independent (non-canonical) and SMAD-dependent (canonical) pathways and is a main inducer of EMT and metastases [54]. Expression of SMAD7 is induced by TGF-β but SMAD7 also acts as a negative regulator by restraining the pathway activity [55]. Using a panel of cancer cells, Yu et al. made the finding that TGF-β increased SMAD7 transcription but not its protein abundance [56••]. The explanation for this interesting observation is that TGF-β activates the expression of miR-182, which suppresses SMAD7 protein. While overexpression of miR-182 supports breast cancer invasion and bone destruction, miR-182 inhibition facilitates TGF-β-mediated increase of SMAD7 protein abundance and prevents TGF-β-induced EMT and cancer cell invasion [56••].

Conclusions

MiRNAs are small non-coding RNAs that regulate various physiological processes and are implicated in disease mechanisms including tumorigenesis and metastases. Recent research provided great insights into the implication of miRNAs in oncogenic signaling and cancer formation (Fig. 1). This research helps to better understand the mechanisms of disease and provides the opportunity for a translational exploitation of miRNAs. For instance, ongoing clinical trials are aimed at investigating the suitability of miRNAs to serve as biomarkers to refine the diagnosis or to improve post-intervention controls. Since miRNAs also have disease-modifying capacities, they may also be useful for therapeutic interventions. This rapidly moving and highly exciting field of science is very likely to further improve our understanding of normal and pathological molecular mechanisms and may perhaps even improve the way cancer patients are treated in the future.

Funding Information The authors were supported by grants from the Deutsche Forschungsgemeinschaft.

Compliance with Ethical Standards

Conflict of Interest E. Hesse declares that he has no conflicts of interest. H. Taipaleenmäki declares that she has no conflicts of interest. Hanna Taipaleenmäki and Eric Hesse report grants from Deutsche Forschungsgemeinschaft and a patent pending.

Human and Animal Rights This article does not contain any studies with human or animal subjects performed by any of the authors.

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56.•• Yu J, Lei R, Zhuang X, Li X, Li G, Lev S, et al. MicroRNA-182 targets SMAD7 to potentiate TGFβ-induced epithelial-mesenchymal transition and metastasis of cancer cells. Nat Commun. 2016;7:13884. https://doi.org/10.1038/ncomms13884 **Describes that TGF-β increases the expression of miR-182, which suppresses SMAD7 protein. Since SMAD7 is a TGF-β antagonist, this mechanism disables the auto inhibition of TGF-β signaling and potentiates TGF-β-induced EMT and cancer cell invasion.**