TRENDS IN SCIENCES 2025; 22(7): 9604
https://doi.org/10.48048/tis.2025.9604
REVIEW ARTICLE

A Review of Advancements in Investigating MicroRNA Roles in Breast Cancer Bone Metastasis

Ali Zainal Abidin[1], Teguh Aryandono[2], Ignatius Raden Endy Widya Putranto[3], Arum Tri Wahyuningsih[4] and Dyah Laksmi Dewi[2,*]

[1]Departement of Molecular Medicine, Master's Program in Biomedical Sciences, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Division of Surgical Oncology, Department of Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3]Department of Histology and Cell Biology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[4] Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

([*]Corresponding author's email: dyah.laksmi.d@ugm.ac.id)

Received: 25 December 2024, Revised: 29 January 2025, Accepted: 5 February 2025, Published: 10 May 2025

Abstract

Breast cancer is among the most prevalent malignancies affecting women globally. Metastasis is a common clinical issue associated with a poor prognosis for breast cancer patients. Bones are one of the most common target organs for metastasis found in breast cancer patients, with a frequency of 70% in metastatic breast cancer. Dysregulation of microRNA (miRNA) is one of the critical molecular factors that mediates bone metastasis in breast cancer. miRNAs expressed by primary breast cancer tissue are known to shape the pre-metastatic bone microenvironment and cause osteolytic bone metastasis in breast cancer. Further investigation is required into the biological implications of alterations in miRNA expression during bone metastasis. In summary, recent advancements in understanding the role of miRNAs in breast cancer bone metastasis have explained the fundamental mechanisms of this process and introduced novel possibilities for improved diagnostic, prognostic, and therapeutic approaches.

Keywords: Biomarker, Breast cancer, Bone metastasis, miRNA, miRNA therapy

Introduction

Breast cancer is among the most prevalent malignancies affecting women globally. Data from GLOBOCAN 2022 reported that there were approximately 2,296,840 cases of breast cancer worldwide, with a mortality rate reaching 666,103 per year. Research conducted by Arnold *et al.* [1] predicts that the global cases of breast cancer will increase by more than 3 million new cases and 1 million deaths by 2040. Metastasis is a common clinical issue associated with poor prognosis of breast cancer patients [2]. Metastasis spreads and develops secondary tumors in surrounding tissues and organs distant from the primary cancer site. Metastasis has become one of the hallmarks of cancer, and it is responsible for the high number of cancer-related deaths, especially in breast cancer [3]. During metastasis development, cancer cells undergo various molecular and epigenetic events that ultimately facilitate the cancer cells to "escape" from the primary cancer tissue [4].

Bones are one of the most common target organs for metastasis found in breast cancer patients, with approximately 70% of breast cancer patients experiencing bone metastasis [2]. Bone metastasis in breast cancer is generally found in the axial skeleton.

Previous study reported that the most common sites of bone metastasis occur in the ribs (69.2%), femur (58.5%), spine (36.9%), and pelvis (23.1%) [5]. Bone metastasis in breast cancer patients is associated with a poor prognosis and a significant decrease in life expectancy of about 2 - 3 years after diagnosis [6].

Bone metastasis in breast cancer is a complex process involving several stages and molecular events. Dysregulation of microRNA (miRNA) is one of the crucial factors that has been linked to the occurrence of bone metastasis in breast cancer. miRNA expressed by primary breast cancer tissues is known to play an essential role in influencing the pre-metastatic bone microenvironment and causing osteolytic bone metastasis in breast cancer [7]. A more comprehensive understanding of the involvement of miRNA dysregulation in the process of bone metastasis in breast cancer is crucial for the development of more effective diagnostic and therapeutic strategies. Therefore, this review will focus on exploring the clinical implications of miRNA dysregulation related to bone metastasis in breast cancer.

Pathophysiology of bone metastasis in breast cancer

Bone is one of the organs that most frequently becomes the site of metastasis. This is due to the bone microenvironment that favors cancer cells to develop and form metastatic colonies, such as the abundant blood supply in the bone matrix and red bone marrow, the presence of adhesive molecules released by cancer cells to bind with stromal cells in the bone marrow, and the increased production of angiogenic factors and bone resorption factors that play a crucial role in facilitating the spread of cancer cells to the bone [8]. Therefore, the composition of the bone microenvironment and its interaction with cancer cells can explain the mechanisms underlying the organotropism of metastasis.

Organotropism or "organ-specific metastasis" is a term used to explain the process where cancer cells tend to metastasize to certain specific organs, and this process is not random. The term organotropism was first introduced by Paget as part of the "seed and soil" hypothesis [9]. Various breast cancer studies have supported this hypothesis, and researchers have explained the genetic basis for cancer colonization in distant organs. In addition, the host's microenvironment and the adaptive processes experienced by cancer cells at metastatic sites play a role in the extravasation and colonization of cancer cells at specific metastatic locations [10]. A brief visualization of the mechanism of bone metastasis in breast cancer can be seen in **Figure 1**.

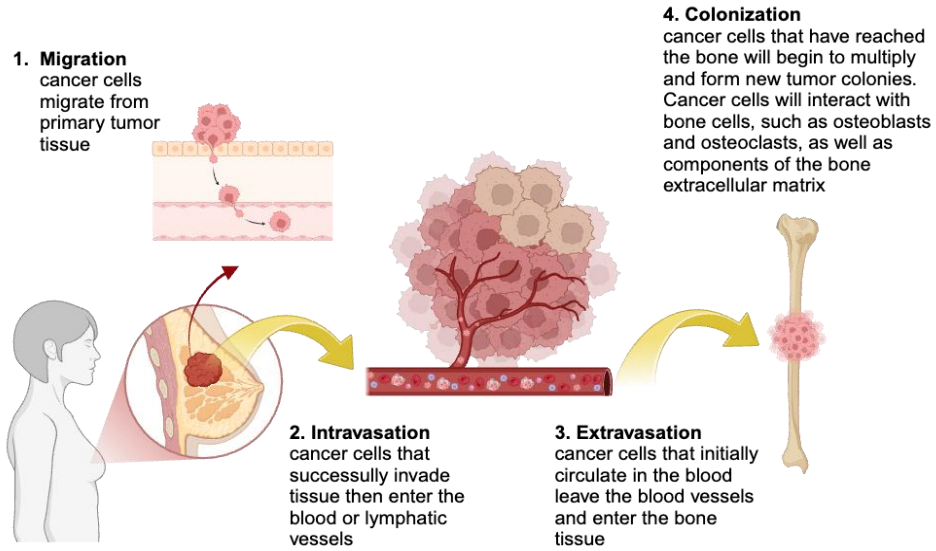

Figure 1 Bone metastasis mechanism in breast cancer [11].
Created in BioRender. Dewi, D. (2025) https://BioRender.com/t08x596

In general, the bone metastasis process can be divided into several stages: The release and spread of cancer cells, adhesion and invasion into the bone, and metastasis in the bone. During the process of transforming normal epithelial cells into breast cancer cells, the adhesion process of basal membrane cells will be disrupted, allowing the release of cancer cells and invasion into the surrounding tissue. At the same time, this process requires cancer cells to exit the microenvironment of their primary tumor tissue and to be able to degrade extracellular matrix proteins. To enter the bloodstream and colonize local metastatic sites, cancer cells must pass through the basement membrane and extracellular matrix. Matrix metalloproteinases (MMP) are a superfamily of several proteinases that can degrade extracellular matrix proteins and enhance the migratory capacity of breast cancer cells towards surrounding tissues and target metastatic organs [12].

Epithelial-mesenchymal transition (EMT) is an essential process in bone metastasis. EMT refers to the ability of cancer cells to change their phenotype from epithelial cells to mesenchymal cells through a series of morphogenetic processes [13]. After breast cancer cells change their phenotype, the cancer cells will initiate the metastasis process by intravasation into the nearest capillaries to facilitate neovascularization for survival. Intravasation is the process of cancer cells spreading to distant organs through the lumen of blood vessels, mediated either actively or passively. This process depends on the type of cancer, the microenvironment, and the blood vessels [14]. Cancer cells located along the edges of blood vessels can disrupt the vascular endothelium through a mechanism mediated by mitosis, after which the cancer cells are released into the circulation [15].

Angiogenesis is one of the abilities of cancer cells to form new blood vessels that will supply nutrients and oxygen to cancer cells, and migrate to the target metastasis organs. The process of angiogenesis involves various factors known as pro-angiogenesis and anti-angiogenesis. In cancer, the process of angiogenesis occurs due to an imbalance between pro-angiogenesis and anti-angiogenesis signals [16]. Angiogenic and growth factors enable cancer cells to invade through the basal membrane, adhere to the endothelial membrane, and pass-through gap junctions to spread into the circulation [17]. Since being in the bone microenvironment, breast cancer cells can release various factors that stimulate bone resorption and angiogenesis, leading to the growth of bone metastases and increasing the attraction of new breast cancer cells to the bone [18].

Breast cancer metastatic cells that have successfully undergone intravasation through the blood or lymphatic circulation will become circulating tumor cells (CTC). In the circulation, CTCs will face many obstacles, including shear forces in circulation, collisions with host cells, and encountering the body's immunity. All of these factors can affect the survival of CTCs and limit their ability to metastasize to distant organs [19]. Most CTCs will be phagocytosed or undergo apoptosis, leaving only a few CTCs that can survive to reach the target metastasis organ. To survive, CTCs must evade the immune system surveillance after detaching from the primary cancer tissue. One of the blood cells that CTCs can exploit after entering circulation is platelet activation. By inducing platelet aggregation, CTCs will be protected from immune system surveillance, experience arrest in blood vessels, and its survival will increase [20].

Adhesion and capture of breast cancer cells on the endothelium is an essential step in the extravasation process mediated by the interaction of multiple ligands and receptors, including selectins, cadherins, integrins, CD44 cell surface adhesion receptors, immunoglobulin superfamily receptors, and also chemokine receptors, such as CXCR4 and CXCR7 [21]. Surviving CTCs will pass through and become trapped in small capillary vessels. This will cause the capillaries to rupture, leading CTCs to undergo the process of transendothelial migration (TEM) and extravasation into the surrounding tissue [22]. Bone has highly permeable sinusoidal vessels, allowing CTCs to metastasize to the bone and liver. Once CTCs reach the surrounding tissues, they can proliferate again to form solid secondary cancer tissues [23].

Target organ colonization is the final step of the metastasis process, involving the ability of cancer cells to form solid secondary cancer tissues. Breast cancer cells that successfully pass through the endothelial barrier and infiltrate the target organ are called disseminated tumor cells (DTC). Even though they have successfully entered the target organ for metastasis, DTCs will still face challenges while adapting to the

new tumor microenvironment [22]. Cancer cells will go through the metastatic dormancy phase during migration to the distant organs. This metastatic dormancy phase occurs as part of the DTCs' adaptation process to the new tumor microenvironment, where the cells reside in the metastatic organ as single cells or micrometastasis foci. In this phase, proliferation and apoptosis occur at the same rate, or the infiltration process is blocked in the G0 phase of the cell cycle. In breast cancer patients who have achieved remission, dormant cancer cells remain in the body and are considered responsible for the recurrence that occurs later [24].

miRNA: biogenesis and function

miRNAs are short non-coding RNA molecules, approximately 18-25 nucleotides in size, known for their role in regulating gene expression at the post-transcriptional level [25]. Each miRNA has several target genes whose expression can be inhibited by binding complementarily to the 3'-untranslated region (3'-UTR) of target mRNAs. After forming a binding complex with the 3'-UTR, miRNA will initiate the inhibition of the translation process or degradation of the mRNA, depending on the complementarity of the binding [26]. Additionally, miRNA can also bind complementarily to the 5'-untranslated region (5'-UTR), coding region, and within the promoter region [27]. miRNA that has bound complementarily to the 5'-UTR and coding region can silence gene expression [28].

So far, it is known that around ± 2,000 miRNAs in humans have been identified and registered in miRBase, and each miRNA has hundreds or even thousands of mRNA targets [29]. miRNA plays an essential role as the primary regulator of about 30% of protein-coding genes in humans and also plays a crucial role in regulating various cellular biological activities, such as proliferation, differentiation, migration, and apoptosis [30]. Genes that encode miRNA are usually localized in non-coding regions or the intron regions of protein-coding genes within the DNA, referred to as intragenic miRNA [31].

The biogenesis of miRNA begins with the transcription of the miRNA-coding gene by RNA polymerase II into primary miRNA (pri-miRNA), which has a structure with a cap and a poly (A) tail. It is also known that RNA polymerase III can produce some pri-miRNAs. Subsequently, pri-miRNA will be cleaved and further processed by the microprocessor complex, which consists of RNAse III Drosha and DiGeorge syndrome critical region 8 (DGCR8), into a stem-loop structure approximately 85 nucleotides long, known as pre-miRNA [32].

Pre-miRNA is then transported from the nucleus to the cytoplasm by the Ran/GTP/Exportin 5 complex and is further processed by RNAse III Dicer by removing the terminal loop, resulting in the formation of mature miRNA duplexes measuring approximately 20 - 25 nucleotides. One strand of the duplex will undergo degradation, and the other strand will function as mature miRNA [32]. After that, the mature miRNA will be transported into a protein complex called RNA-induced silencing complex (RISC), which also involves argonaute proteins and GW182. RISC can bind to the 3'-UTR end of the target mRNA. The formation of a complementary and perfect binding between miRNA and the 3'-UTR end of the target mRNA can lead to the degradation of the target mRNA. On the other hand, if a complementary but imperfect binding occurs, it will inhibit the translation process [33]. A brief visualization of miRNA biogenesis can be seen in **Figure 2**.

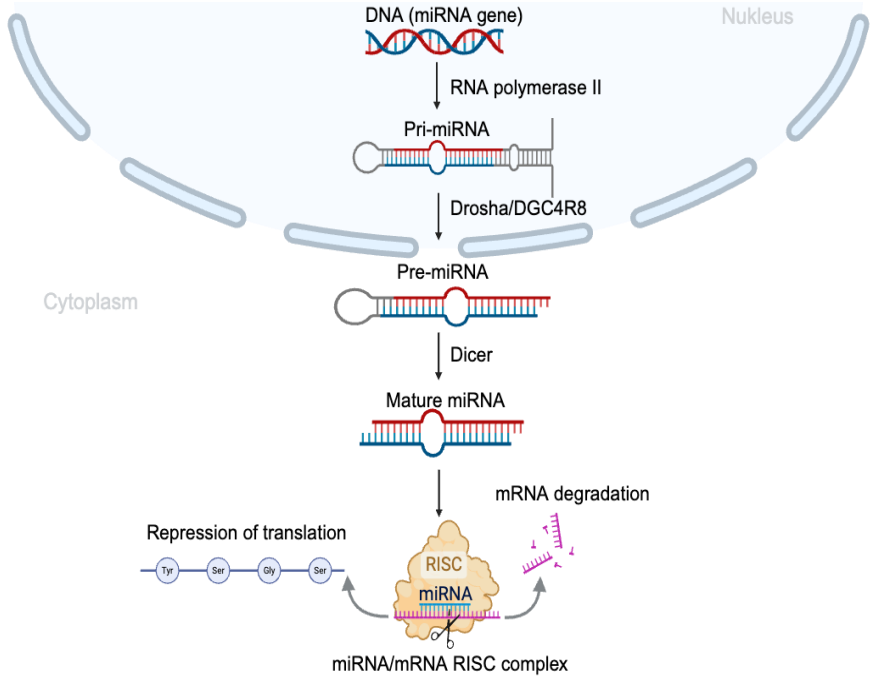

Figure 2 miRNA biogenesis [32]. Created in BioRender. Dewi, D. (2025) https://BioRender.com/s83j389.

The role of miRNA in breast cancer bone metastasis

The expression of miRNA in breast cancer shows specific and distinct patterns compared to normal breast tissue, indicating that miRNA has a potential role as a biomarker for the diagnosis and prognosis of breast cancer [34]. With the advancement of technology in the field of molecular biology, several methods have been developed to comprehensively analyze miRNA expression, leading to new findings regarding the role of miRNA in the oncogenesis of breast cancer, including its involvement in metastasis to visceral organs and the mechanisms of resistance to hormonal therapy [35]. Several breast cancer subtypes exhibit distinct and specific miRNA expression patterns [36]. A brief visualization of the mechanisms of miRNAs in breast cancer bone metastasis can be seen in **Figure 3**.

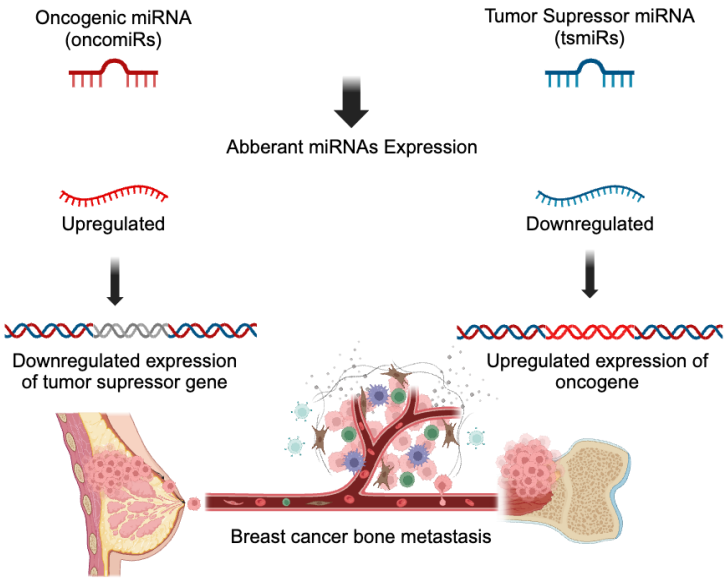

Figure 3 Regulatory mechanisms of oncogenic and tumor suppressor miRNAs in breast cancer bone metastasis [37]. Created in BioRender. Dewi, D. (2025) https://BioRender.com/i70c694.

Dysregulation of miRNA can lead to changes in miRNA expression, especially in cancer. Research results report that more than half of human miRNA genes are located in genomic regions involved in cancer [38,39]. Based on their role in oncogenesis, miRNAs can be classified into oncogenic miRNAs (oncomiRs) and tumor suppressor miRNAs (tsmiRs). Dysregulation of miRNAs in cancer generally occurs in oncomiRs that exhibit increased expression (upregulation) and in tsmiRs that exhibit downregulation, which are known to play essential roles in the mechanisms of oncogenesis [40]. It is also known that miRNA is classified based on their role in metastasis, namely pro-metastatic miRNA (metastamiR) and metastasis-suppressor miRNA [41].

miRNA expressed by primary breast cancer tissue is known to play an essential role in influencing the pre-metastatic bone microenvironment and causing bone metastasis in breast cancer. Metastatic breast cancer cells in the bone modify the bone microenvironment and facilitate the proliferation and dissemination of more cancer cells [42]. miRNAs produced by primary cancer cells can alter the bone stromal cells, including osteoblasts and osteoclasts, to establish a nutrient and growth factor-rich environment that facilitates the proliferation of cancer cells [43]. miRNAs also regulate the chemotaxis of cancer cells, which is their capacity to migrate toward specific chemical stimuli. Cancer cells that have separated from the primary breast cancer tissue will respond to chemotactic signals generated by the bone microenvironment, and this process is partially regulated by miRNA expression [44]. Moreover, miRNAs can modulate the expression of adhesion molecules on cancer cell surfaces, thus impacting the capacity of cancer cells to engage with the bone extracellular matrix and migrate through the tissue.

Metastatic cancer cells in the bone frequently avoid the immune system. miRNAs can help cancer cells evade the immune system through several mechanisms, such as suppressing the immune responses or enhancing the resistance of cancer cells to chemotherapy [45]. miRNAs have crucial regulatory roles in the interaction between cancer cells and the bone microenvironment, orchestrating several cell types, signaling molecules, and extracellular components. For example, miRNAs can modulate the secretion of growth factors by stromal cells, thus promoting the proliferation of cancer cells. Furthermore, miRNAs produced by cancer cells can interact with miRNAs generated by bone stromal cells, establishing an intricate regulatory network [46].

Several miRNAs have been identified as key regulators in the bone metastases of breast cancer. MiR-21 is one of the oncomiRs that is overexpressed in several cancers and has been associated with very poor prognosis in cancer patients. In breast cancer, miR-21 promotes bone metastasis. The excessive expression of miR-21 in breast cancer cells is stimulated by lysophosphatidic acid (LPA1) through the LPA1/PI3K/ZEB1 signaling pathway [47]. MiR-940 is one type of oncomiR secreted by breast cancer cells, which is known to play a crucial role in inducing osteoblastic bone metastasis and is responsible for the bone remodeling modification process during the metastasis process [48]. A brief visualization of the roles of various miRNAs mediating the metastasis process from breast tissue to bone can be seen in **Figure 4**.

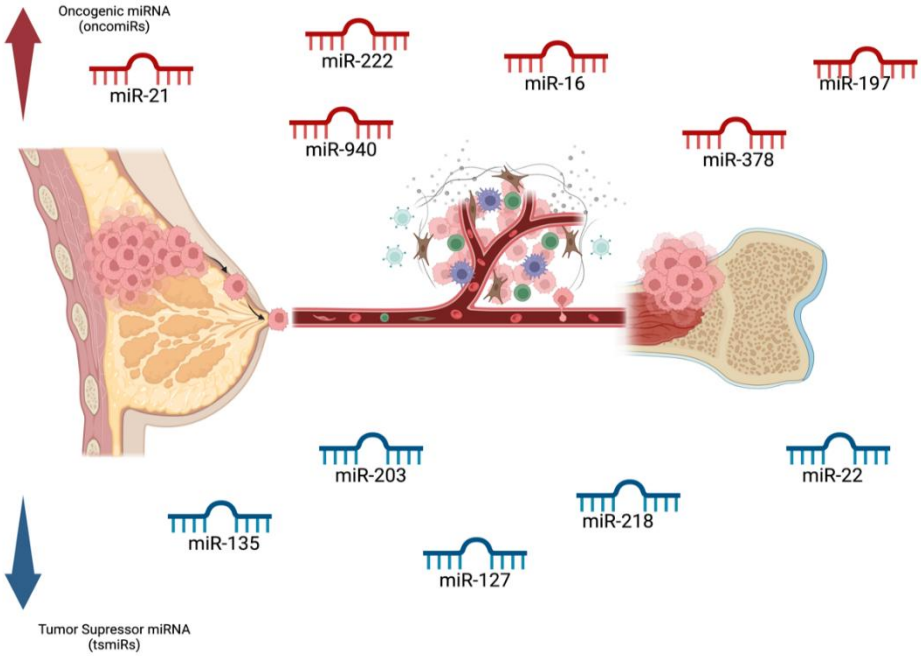

Figure 4 Recent findings in the roles of microRNAs in breast cancer bone metastasis. Multiple miRNAs undergo alterations in their expression levels and influence tumor cells metastasis from breast cancer tissue to the bone [49]. Created in BioRender. Dewi, D. (2025) https://BioRender.com/g61m808.

A previous study indicated that breast cancer cells can release exosomes containing miRNA-19a to pre-osteoclast cells and subsequently inhibit PTEN expression. The decrease in PTEN expression induces the activity of the NFκB and AKT signaling pathways, which promote osteoclast differentiation and bone resorption. The absorption of exosomes containing miR-19a by preosteoclast cells depends on the concentration of exosomes; the higher the concentration of exosomes, the more miR-19a will be absorbed by the preosteoclast cells. Integrin-binding sialoprotein (IBSP) released by estrogen receptor (ER) positive breast cancer cells plays a role in attracting preosteoclast cells into the bone microenvironment and facilitating the regulation of miR-19a in the bone resorption process [50].

miR-16, miR-133a, and miR-223 are also known to play a role in the occurrence of bone metastasis in breast cancer patients. In breast cancer, the increased expression of miR-16 is involved in bone metastasis by enhancing osteoclast function and increasing bone damage by upregulating RANKL, IL-1β, PTHrP, and other factors. The expression of miR-133a and miR-223 also increases in breast cancer, but they inhibit bone damage by reducing the expression of osteolytic factors, thereby decreasing osteoclast activity. Further research is needed to precisely determine the role of miR-16 in the pathophysiology of bone metastasis in breast cancer [51].

A previous study reported that two osteoclast miRNAs, miRNA-16 and miRNA-378, play a role in the osteoclast formation process, and their expression is increased in the serum of breast cancer patients with bone metastasis. In addition to oncomiRs, it is also known that tsmiRs are involved in the occurrence of bone metastasis in breast cancer. For example, miR-135 and miR-203 are two tsmiRs frequently found in breast cancer with bone metastasis. In the breast, the expression of miR-135 and miR-203 increases in normal breast epithelial cells. In contrast, the expression of those miRNAs decreases in bone metastasis specimens of breast cancer patients compared to the normal bone specimens [52]. The expression of miR-135 and miR-203 is known to play a role in protecting the bone from the formation of osteolytic lesions in metastasis animal model by directly targeting RUNX2 in breast cancer cell line MDA-MB-231 [53]. The results of this study suggest that miR-135 and miR-203 may act as tumor suppressors in the occurrence of bone metastasis in breast cancer.

miR-218 is known to play a role in regulating osteomimicry activity, which can also be referred to as "osteo-miR." miR-218 is involved in controlling bone

formation through the upregulation of RUNX2 and the downregulation of Wnt signaling inhibitors (sclerostin, dickkopf-2, and secreted frizzled-related protein 2) in osteoblasts. miR-218 is also recognized as a pro-metastatic miRNA that works by stimulating the expression of Wnt-related proteins (bone sialoprotein and osteopontin) in breast cancer cells that metastasize to the bone. Additionally, miR-218 can facilitate bone metastasis in MDA-MB-231 breast cancer cells by upregulating CXCR4. This chemokine receptor supports the migration of breast cancer cells to bone and mediates cancer cell growth in bone [54].

Several studies have reported that bone metastasis can recur in breast cancer patients within ten years after remission. This indicates that cancer cells can remain dormant within the patient's body for a prolonged period [55]. Several molecules and factors, including miRNAs, play essential roles in the dormancy process of cancer cells that retain the ability to metastasize [56]. Some research findings report that miR-127, miR-197, and miR-222 also play important roles in the dormancy of metastatic breast cancer cells [57].

Table 1 Summary of studies investigating the roles of miRNAs in breast cancer bone metastasis.

Author	Experimental model/ Sample types	Methods	miRNAs	Pattern of expression	Target genes	Potential roles of miRNAs
[47]	- MDA-MB-231 and Hs 578T cell lines - BALB/C nude mice	miRNA microarray qRT-PCR	miR-21	Upregulated	- ZEB1	MicroRNA miR-21 has been classified as an oncomiR, facilitating metastasis in several cancer types. Micro-RNA miR-21 was identified as one of the most significantly upregulated miRNAs in these cells after LPA activation. The functional interplay between ZEB1 and miR-21, which facilitates LPA-dependent metastasis via LPA1 in breast cancers, could significantly influence the identification of a possible target for the development of novel adjuvant medicines aimed at preventing metastatic recurrences in triple-negative breast cancer patients.
[48]	- MDA-MB-231-Luc - UCB408E6E7TERT-33 cell lines - BALB/cAJcl-nu/nu mice	miRNA microarray qRT-PCR	miR-940	Upregulated	- ARHGAP1 - FAM134A	miR-940 enhanced resistance to many chemotherapeutic agents, such as methotrexate and vinblastine. This study demonstrates that a cancer-secreted miRNA induces osteoblastic-type bone metastases, acting as an osteotropic factor in the bone microenvironment.
[50]	- MDA-MB-231 - MCF7, T47D and RAW 264.7 - The athymic-nu/nu mice - Serum samples from early and advanced breast cancer patients	miRNA microarray, qRT-PCR	miR-19a	Upregulated	- PTEN	miR-19a and IBSP acts as mediators for intercellular communication between cancer cells and osteoclasts. This study elucidates the molecular basis of bone metastasis in ER+ breast cancer, necessitating further exploration of the miR19a-IBSP axis for the formulation of an effective therapeutic strategy.

Author	Experimental model/ Sample types	Methods	miRNAs	Pattern of expression	Target genes	Potential roles of miRNAs
[51]	- MDA-MB-231 cell line - RAW264.7 cell line - BALB/c nude mice	qRT- PCR	miR-16 miR-133a miR-223	Upregulated (miR-16); Downregulated (miR-133a and miR-223)	- RANKL - IL-1β - IL-6 - PTHrP - TNF-α	miR-16 may exacerbate bone degradation by promoting osteoclast activity through increased expression of osteoclast differentiation markers and osteolytic factors, whereas miR-133a and miR-223 may inhibit this process. Further research is required to further understand the effect of miRNAs on cancer bone metastasis, the 3 miRNAs could potentially serve as biomarkers and therapeutic targets for breast cancer bone metastasis patients.
[52]	- Serum samples from breast cancer patients with bone metastasis, without bone metastasis and healthy individuals	qRT-PCR	miR-16, miR-378	Upregulated	- RANKL - GM-CSF - MIP-1α, - PTHrP, - IL-8, - IL-6	Osteoclast miRNAs, including miR-16 and miR-378, are prospective targets for treating abnormal osteoclast activity, such as osteolytic bone metastases. The increased levels of miR-16 and miR-378 in bone lesions and serum samples of patients with bone metastases suggest their potential utility as biomarkers for metastasis.
[53]	- Breast cancer tissues - MCF-10A - MCF-7 - MDA-MB-231	qRT-PCR	miR-135 miR-203	Downregulated	- RUNX2	miR-135 and miR-203 act as inhibitors of breast cancer bone metastases *in vivo* by directly targeting the bone-associated transcription factor RUNX2. miR-135 and miR-203 may function as biomarkers and therapeutic targets to impede the advancement of bone metastasis in breast cancer patients.
[54]	- MC3T3-E1 cell line - MDA-MB-231 cell line	qRT- PCR	miR-218	Upregulated	- BSP - OPN - CXCR4 - TCF-1 - β-catenin, - Runx2	miR-218 enhances Wnt activity and the aberrant expression of osteoblastic genes, facilitating the homing and proliferation of breast cancer cells to the bone. The miR-218/Wnt signaling pathway enhances the osteoblast and breast cancer cell activity associated osteomimicry in bone metastasis.

The potential clinical implications of miRNAs in breast cancer bone metastasis

The expression of several miRNAs has been reported to be drivers of molecular alterations in cells during bone metastasis in breast cancer [58]. Over the past decades, many studies have reported the potential of circulating miRNAs in providing clinical roles as diagnostic biomarkers, prognostic biomarkers, and therapeutic targets in breast cancer patients with bone metastasis [59]. In addition, miRNAs can also be potential biomarkers to predict tumor cell responses to certain chemotherapeutic agents [60]. One of the advantages of miRNAs is that they can be detected from formalin-fixed paraffin-embedded (FFPE) tissue blocks and liquid biopsies, such as blood, serum, urine, saliva, and milk fluids [61]. A brief visualization of the

potential clinical implications of miRNAs in breast cancer bone metastasis can be seen in **Figure 5**.

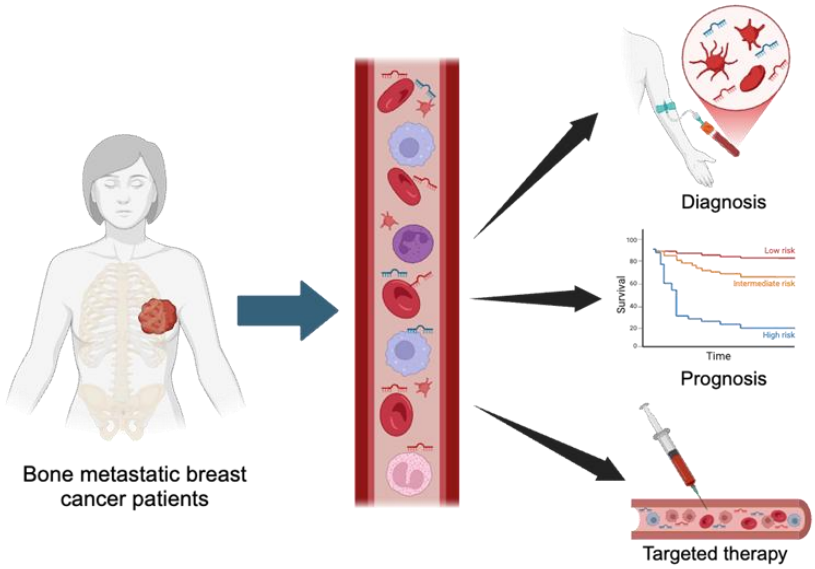

Figure 5 The potential clinical implications of miRNAs in breast cancer bone metastasis [62]. Created in BioRender. Dewi, D. (2025) https://BioRender.com/w94q012

The ability to detect circulating miRNA in blood and body fluids can provide an alternative for early non-invasive diagnosis [62]. The miRNA expression from FFPE tissue blocks that can be used as diagnostic biomarkers is miR-429 for bone metastasis in breast cancer [63]. The increased expression of miRNA-30b-5p in peripheral blood samples from breast cancer patients with bone metastasis has the potential to become a diagnostic biomarker in non-invasive liquid biopsies. Further research is needed to precisely understand the role of miRNA-30b-5p in predicting the occurrence of bone metastasis in breast cancer patients [64].

Furthermore, increased expression of miR-21 is associated with poor prognosis in breast cancer patients with bone metastasis, making miR-21 a potential prognostic biomarker in breast cancer patients with bone metastasis [47]. The development of miRNA-based cancer therapies focuses on restoring the normal regulation of miRNA functions [65]. miRNA-based therapies can use two types of synthetic molecules specifically designed to mimic the functions of natural miRNA. miRNA mimics can be used to restore the function of endogenous miRNA and tumor suppressor miRNA expression, while antagomiR is specifically used to inhibit the overexpressed oncomiR in cancer cells [66]. Further research on the molecular mechanisms of miRNAs in regulating the occurrence of breast cancer metastasis to bone is essential to provide new insights for the development of new therapies for breast cancer patients with bone metastasis.

Challenges and future directions

Research in molecular biology and oncology increasingly highlights miRNA and its role in developing bone metastasis in breast cancer. While there is mounting evidence regarding the role of miRNA in breast cancer bone metastasis, further research is still required. Further investigation into the biological implications of alterations in miRNA expression during the metastatic dissemination of tumor cells from primary breast cancer tissue to bone is still required. Furthermore, investigating the interplay among several miRNAs associated with breast cancer and bone metastasis is crucial for comprehensively understanding the complex mechanisms that drive the progression of bone metastasis in breast cancer. Additionally, investigating the interactions among various miRNAs

may facilitate the advancement of more precise diagnostic and prognostic tools and more effective and comprehensive therapeutic targeting modalities for breast cancer patients with bone metastases. Integrating scientific discoveries about the function of miRNAs in bone metastasis in breast cancer patients into clinical practice can improve quality of life and patients' survival.

Conclusions

Recent advances in miRNA research have promoted a comprehensive understanding of the roles of these molecules in breast cancer bone metastases. MiRNAs play a crucial role in the pathogenesis of breast cancer bone metastasis by regulating genes that promote the dissemination of breast cancer cells to the bone. Moreover, miRNAs have shown potential as biomarkers for the detection of bone metastasis in breast cancer and for predicting patients' prognosis. Additionally, targeting specific miRNAs may become a promising therapeutic approach to inhibit disease progression. In summary, recent advancements in understanding the role of miRNAs in breast cancer bone metastasis have explained the fundamental mechanisms of this condition and introduced novel possibilities to improve diagnostic, prognostic, and therapeutic approaches.

Acknowledgments

This work was supported by the grant from the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research and Technology, Republic of Indonesia (048/E5/PG.02.00.PL/2024;2645/UNl/DITLIT/PT.01.03/2024). We also thank the Precision Oncology-Based Omics Research Collaboration Center (PKR-PrOmics) for providing mentoring services.

References

[1] M Arnold, E Morgan, H Rumgay, A Mafra, D Singh, M Laversanne, J Vignat, JR Gralow, F Cardoso, S Siesling and I Soerjomataram. Current and future burden of breast cancer: Global statistics for 2020 and 2040. *The Breast* 2022; **66**, 20.

[2] S Wang, W Wu, X Lin, KM Zhang, QL Wu, M Luo and J Zhou. Predictive and prognostic biomarkers of bone metastasis in breast cancer: Current status and future directions. *Cell & Bioscience* 2023; **13(1)**, 224.

[3] J Fares, MY Fares, HH Khachfe, HA Salhab and Y Fares. Molecular principles of metastasis: A hallmark of cancer revisited. *Signal Transduction and Targeted Therapy* 2020; **5(1)**, 28.

[4] D Yang, SK Denny, PG Greenside, AC Chaikovsky, JJ Brady, Y Ouadah, JM Granja, NS Jahchan, JS Lim, S Kwok, CS Kong, AS Berghoff, A Schmitt, HC Reinhardt, KS Park, M Preusser, A Kundaje, WJ Greenleaf, J Sage and MM Winslow. Intertumoral heterogeneity in SCLC is influenced by the cell type of origin. *Cancer Discovery* 2018; **8(10)**, 1316-1331.

[5] KB Siregar and MA Anas. Unveiling bone metastasis: Exploring histological subtypes of breast cancer in Indonesia's tertiary referral hospital. *Cancer Treatment and Research Communications* 2023; **37**, 100764.

[6] C Tulotta and P Ottewell. The role of IL-1B in breast cancer bone metastasis. *Endocrine-Related Cancer* 2018; **25(7)**, R421-R434.

[7] X Yuan, N Qian, S Ling, Y Li, W Sun, J Li, R Du, G Zhong, C Liu, G Yu, D Cao, Z Liu, Y Wang, Z Qi, Y Yao, F Wang, J Liu, S Hao, X Jin and Y Zhao. Breast cancer exosomes contribute to pre-metastatic niche formation and promote bone metastasis of tumor cells. *Theranostics* 2021; **11(3)**, 1429-1445.

[8] AC Chiang and J Massagué. Molecular basis of metastasis. *New England Journal of Medicine* 2008; **359(26)**, 2814-2823.

[9] RR Langley and IJ Fidler. The seed and soil hypothesis revisited-The role of tumor-stroma interactions in metastasis to different organs. *International Journal of Cancer* 2011; **128(11)**, 2527-2535.

[10] PI Croucher, MM McDonald and TJ Martin. Bone metastasis: The importance of the neighbourhood. *Nature Reviews Cancer* 2016; **16(6)**, 373-386.

[11] AN Guerrieri, M Montesi, S Sprio, R Laranga, L Mercatali, A Tampieri, DM Donati and E Lucarelli. Innovative options for bone metastasis treatment: An extensive analysis on biomaterials-based strategies for orthopedic

surgeons. *Frontiers in Bioengineering and Biotechnology* 2020; **8**, 589964.

[12] S Mustafa, S Koran and L AlOmair. Insights into the role of matrix metalloproteinases in cancer and its various therapeutic aspects: A review. *Frontiers in Molecular Biosciences* 2022; **9**, 896099.

[13] X Song, C Wei and X Li. The signaling pathways associated with breast cancer bone metastasis. *Frontiers in Oncology* 2022; **12**, 855609.

[14] N Reymond, BB d'Água and AJ Ridley. Crossing the endothelial barrier during metastasis. *Nature Reviews Cancer* 2013; **13(12)**, 858-870.

[15] AD Wong and PC Searson. Mitosis-mediated intravasation in a tissue-engineered tumor-microvessel platform. *Cancer Research* 2017; **77(22)**, 6453-6461.

[16] BM Hussen, ST Abdullah, MF Rasul, A Salihi, S Ghafouri-Fard, HJ Hidayat and M Taheri. MicroRNAs: An important player in breast cancer angiogenesis and therapeutic targets. *Frontiers in Molecular Biosciences* 2021; **8**, 764025.

[17] RK Kanchan, JA Siddiqui, S Mahapatra, SK Batra and MW Nasser. microRNAs orchestrate pathophysiology of breast cancer brain metastasis: Advances in therapy. *Molecular Cancer* 2020; **19**, 29.

[18] G Van der Pluijm, C Löwik and S Papapoulos. Tumour progression and angiogenesis in bone metastasis from breast cancer: new approaches to an old problem. *Cancer Treatment Reviews* 2000; **26(1)**, 11-27.

[19] M Sereno, M Videira, I Wilhelm, IA Krizbai and MA Brito. miRNAs in health and disease: A focus on the breast cancer metastatic cascade towards the brain. *Cells* 2020; **9(8)**, 1790.

[20] B Strilic and S Offermanns. Intravascular survival and extravasation of tumor cells. *Cancer Cell* 2017; **32(3)**, 282-293.

[21] C Alix-Panabières and K Pantel. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. *Cancer Discovery* 2016; **6(5)**, 479-491.

[22] J Massagué and AC Obenauf. Metastatic colonization by circulating tumour cells. *Nature* 2016; **529(7586)**, 298-306.

[23] YA Fouad and C Aanei. Revisiting the hallmarks of cancer. *American Journal of Cancer Research* 2017; **7**, 1016-1036.

[24] FG Giancotti. Mechanisms governing metastatic dormancy and reactivation. *Cell* 2013; **155(4)**, 750-764.

[25] M Ha and VN Kim. Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology* 2014; **15(8)**, 509-524.

[26] JJ Ipsaro and L Joshua-Tor. From guide to target: Molecular insights into eukaryotic RNA-interference machinery. *Nature Structural & Molecular Biology* 2015; **22(1)**, 20-28.

[27] W Xu, A San Lucas, Z Wang and Y Liu. Identifying microRNA targets in different gene regions. *BMC Bioinformatics* 2014; **15(7)**, S4.

[28] J Zhang, W Zhou, Y Liu, T Liu, C Li and L Wang. Oncogenic role of microRNA-532-5p in human colorectal cancer via targeting of the 5'UTR of RUNX3. *Oncology Letters* 2018; **15(5)**, 7215-7220.

[29] J Alles, T Fehlmann, U Fischer, C Backes, V Galata, M Minet, M Hart, M Abu-Halima, FA Grässer, HP Lenhof, A Keller and E Meese. An estimate of the total number of true human miRNAs. *Nucleic Acids Research* 2019; **47(7)**, 3353-3364.

[30] Y Shi, Z Liu, Q Lin, Q Luo, Y Cen, J Li, X Fang and C Gong. MiRNAs and cancer: Key link in diagnosis and therapy. *Genes* 2021; **12(8)**, 1289.

[31] K Saliminejad, HR Khorram Khorshid, SS Fard and SH Ghaffari. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. *Journal of Cellular Physiology* 2019; **234(5)**, 5451-5465.

[32] Y Peng and CM Croce. The role of MicroRNAs in human cancer. *Signal Transduction and Targeted Therapy* 2016; **1**, 15004.

[33] H Iwakawa and Y Tomari. Life of RISC: Formation, action, and degradation of RNA-induced silencing complex. *Molecular Cell* 2022; **82(1)**, 30-43.

[34] AA Yousif, HA Eisa, AM Nawar, MS Abd El-latif and EG Behiry. Study of serum microRNA-99a relative expression as a diagnostic and prognostic noninvasive biomarker of breast cancer in

Egyptian females. *Gene Reports* 2020; **19**, 100593.

[35] PMM Ozawa, E Vieira, DS Lemos, ILM Souza, SM Zanata, VC Pankievicz, TR Tuleski, EM Souza, PF Wowk, CA Urban, F Kuroda, RS Lima, RC Almeida, DF Gradia, IJ Cavalli, LR Cavalli, D Malheiros and EMSF Ribeiro. Identification of miRNAs enriched in extracellular vesicles derived from serum samples of breast cancer patients. *Biomolecules* 2020; **10(1)**, 150.

[36] RP Arun, HF Cahill and P Marcato. Breast cancer subtype-specific miRNAs: Networks, impacts, and the potential for intervention. *Biomedicines* 2022; **10(3)**, 651-651.

[37] M Saikia, S Paul and S Chakraborty. Role of microRNA in forming breast carcinoma. *Life Sciences* 2020; **259**, 118256.

[38] GA Calin, C Sevignani, CD Dumitru, T Hyslop, E Noch, S Yendamuri, M Shimizu, S Rattan, F Bullrich, M Negrini and CM Croce. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proceedings of the National Academy of Sciences of the United States of America* 2004; **101(9)**, 2999-3004.

[39] EV Schooneveld, H Wildiers, I Vergote, PB Vermeulen, LY Dirix and SJV Laere. Dysregulation of microRNAs in breast cancer and their potential role as prognostic and predictive biomarkers in patient management. *Breast Cancer Research* 2015; **17**, 21.

[40] AA Svoronos, DM Engelman and FJ Slack. OncomiR or tumor suppressor? The duplicity of microRNAs in cancer. *Cancer research* 2016; **76(13)**, 3666-3670.

[41] R Baffa, M Fassan, S Volinia, B O'Hara, CG Liu, JP Palazzo, M Gardiman, M Rugge, LG Gomella, CM Croce and A Rosenberg. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. *The Journal of Pathology* 2009; **219(2)**, 214-221.

[42] MC Sanmartin, FR Borzone, MB Giorello, N Pacienza, G Yannarelli and NA Chasseing. Bone marrow/bone pre-metastatic niche for breast cancer cells colonization: The role of mesenchymal stromal cells. *Critical Reviews in Oncology/Hematology* 2021; **164**, 103416.

[43] L Sui, J Wang, WG Jiang, X Song and L Ye. Molecular mechanism of bone metastasis in breast cancer. *Frontiers in Oncology* 2024; **14**, 1401113.

[44] HM Nail, CC Chiu, CH Leung, MMM Ahmed and HMD Wang. Exosomal miRNA-mediated intercellular communications and immunomodulatory effects in tumor microenvironments. *Journal of Biomedical Science* 2023; **30(1)**, 69.

[45] K Kousar, T Ahmad, MS Abduh, B Kanwal, SS Shah, F Naseer and S Anjum. miRNAs in regulation of tumor microenvironment, chemotherapy resistance, immunotherapy modulation and miRNA therapeutics in cancer. *International Journal of Molecular Sciences* 2022; **23(22)**, 13822.

[46] LN Barrera, PM Ridley, C Bermejo-Rodriguez, E Costello and PA Perez-Mancera. The role of microRNAs in the modulation of cancer-associated fibroblasts activity during pancreatic cancer pathogenesis. *Journal of Physiology and Biochemistry* 2022; **79(1)**, 193-204.

[47] D Sahay, R Leblanc, TGP Grunewald, A Srikant, J Ribeiro, C Philippe and P Olivier. The LPA1/ZEB1/miR-21-activation pathway regulates metastasis in basal breast cancer. *Oncotarget* 2015; **6(24)**, 20604-20620.

[48] K Hashimoto, H Ochi, S Sunamura, N Kosaka, Y Mabuchi, T Fukuda, K Yao, H Kanda, K Ae, A Okawa, C Akazawa, T Ochiya, M Futakuchi, S Takeda and S Sato. Cancer-secreted hsa-miR-940 induces an osteoblastic phenotype in the bone metastatic microenvironment via targeting ARHGAP1 and FAM134A. *Proceedings of the National Academy of Sciences* 2018; **115(9)**, 2204-2209.

[49] MT Haider, DJ Smit and H Taipaleenmaeki. MicroRNAs: Emerging regulators of metastatic bone disease in breast cancer. *Cancers* 2022; **14(3)**, 729.

[50] K Wu, J Feng, F Lyu, F Xing, S Sharma, Y Liu, SY Wu, D Zhao, A Tyagi, RP Deshpande, X Pei, MG Ruiz, H Takahashi, S Tsuzuki, T Kimura, YY Mo, Y Shiozawa, R Singh and K Watabe. Exosomal miR-19a and IBSP cooperate to induce osteolytic bone metastasis of estrogen receptor-

positive breast cancer. *Nature Communications* 2021; **12(1)**, 5196.

[51] K Kitayama, T Kawamoto, Y Kawakami, H Hara, T Takemori, S Fujiwara, Y Shunsuke, T Miyamoto, M Yutaka, Y Hoshino, K Kenichiro, T Matsumoto, T Matsushita, N Takahiro, R Kuroda and A Toshihiro. Regulatory roles of miRNAs 16, 133a, and 223 on osteoclastic bone destruction caused by breast cancer metastasis. *International Journal of Oncology* 2021; **59(5)**, 97.

[52] B Ell, L Mercatali, T Ibrahim, N Campbell, H Schwarzenbach, K Pantel, D Amadori and Y Kang. Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis. *Cancer Cell* 2013; **24(4)**, 542-556.

[53] H Taipaleenmäki, G Browne, J Akech, J Zustin, AJV Wijnen, JL Stein, E Hesse, GS Stein and JB Lian. Targeting of Runx2 by miR-135 and miR-203 impairs progression of breast cancer and metastatic bone disease. *Cancer Research* 2015; **75(7)**, 1433-1444.

[54] MQ Hassan, Y Maeda, H Taipaleenmaki, W Zhang, M Jafferji, JAR Gordon, Z Li, CM Croce, AJV Wijnen, JL Stein, GS Stein and JB Lian. miR-218 directs a Wnt signaling circuit to promote differentiation of osteoblasts and osteomimicry of metastatic cancer cells. *Journal of Biological Chemistry* 2012; **287(50)**, 42084-42092.

[55] M Ono, N Kosaka, N Tominaga, Y Yoshioka, F Takeshita, R Takahashi, M Yoshida, H Tsuda, J Tamura and T Ochiya. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. *Science Signaling* 2014; **7(332)**, ra63.

[56] MH Younis, L Fuentes-Rivera, S Summers and J Pretell-Mazzini. Survival in patients with carcinomas presenting with bone metastasis at diagnosis: A SEER population-based cohort study. *Archives of Orthopaedic and Trauma Surgery* 2020; **141(3)**, 367-373.

[57] PK Lim, SA Bliss, SA Patel, M Taborga, MA Dave, LA Gregory, SJ Greco, M Bryan, PS Patel and R Pranela. Gap junction - mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. *Cancer Research* 2011; **71(5)**, 1550-1560.

[58] M Puppo, H Taipaleenmäki, E Hesse and P Clézardin. Non-coding RNAs in bone remodelling and bone metastasis: Mechanisms of action and translational relevance. *British Journal of Pharmacology* 2019; **178(9)**, 1936-1954.

[59] Q Tang, H Ouyang, D He, C Yu and G Tang. MicroRNA-based potential diagnostic, prognostic and therapeutic applications in triple-negative breast cancer. *Artificial Cells, Nanomedicine, and Biotechnology* 2019; **47(1)**, 2800-2809.

[60] TH Vo, EL Esam, E Jordan, O O'Donovan, EA McNeela, JP Mehta and S Rani. miRNAs as biomarkers of therapeutic response to HER2-targeted treatment in breast cancer: A systematic review. *Biochemistry and Biophysics Reports* 2024; **37**, 101588.

[61] BN Hannafon and WQ Ding. miRNAs as biomarkers for predicting the progression of ductal carcinoma *in situ*. *The American Journal of Pathology* 2018; **188(3)**, 542-549.

[62] B Ortiz-Quintero. Cell-free microRNAs in blood and other body fluids, as cancer biomarkers. *Cell Proliferation* 2016; **49(3)**, 281-303.

[63] X Zhang, X Yu, Z Zhao, Z Yuan, P Ma, Z Ye, L Guo, S Xu, L Xu, T Liu, H Liu and S Yu. MicroRNA-429 inhibits bone metastasis in breast cancer by regulating CrkL and MMP-9. *Bone* 2020; **130**, 115139.

[64] H Estevão-Pereira, J Lobo, S Salta, M Amorim, P Lopes, M Cantante, B Reis, L Antunes, F Castro, S Palma, CS Gonçalves, BM Costa, R Henrique and C Jerónimo. Overexpression of circulating MiR-30b-5p identifies advanced breast cancer. *Journal of Translational Medicine* 2019; **17**, 435.

[65] J Szczepanek, M Skorupa and A Tretyn. MicroRNA as a potential therapeutic molecule in cancer. *Cells* 2022; **11(6)**, 1008.

[66] Z Fu, L Wang, S Li, F Chen, KKW Au-Yeung, and C Shi. MicroRNA as an Important Target for Anticancer Drug Development. *Frontiers in Pharmacology* 2021; **12**, 736323.