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The impacts of exosomes on bone metastatic progression and their potential clinical utility

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ABSTRACT

Bone is one of the most common sites of cancer metastasis. Once cancer metastasizes to the bone, the mortality rate of cancer patients dramatically increases. Although the exact mechanisms for this observation remain elusive, recent studies have revealed that the complex crosstalk between bone marrow microenvironment and bone metastatic cancer cells is responsible for the induction of treatment resistance. Consequently, bone metastasis is currently considered incurable. Bone metastasis not only impairs the patients' survival, but also negatively affects their quality of life by causing painful complications. It has recently been implicated the regulatory role of exosomes in cancer development and/or progression as a delivery biomaterial between cancer cells and tumor microenvironment. However, little is known as to how exosomes contribute to the progression of bone metastasis by impaction on the crosstalk between bone metastatic cancer cells and bone marrow microenvironment. Here, we highlighted the emerging roles of cancer-derived exosomes in (i) the process of dissemination and bone colonization of bone metastatic cancer cells, (ii) the enhancement of crosstalk between bone marrow microenvironment and bone metastatic cancer cells, (iii) the development of its resultant painful complications, and (iv) the clinical applications of exosomes in the bone metastatic setting.

1. Introduction

Bone metastasis is devastating for cancer patients as in the United States alone, about 350,000 of them die each year from this disease progression (Huang et al., 2020). Additionally, in prostate and breast cancer, which are both known to metastasize to the bone, the median survival time of patients with bone metastases is 25 and 27 months, respectively, with current cancer patients considered incurable when they develop bone metastasis (Huang et al., 2020). However, bone metastases are not only incurable; they also compromise patients' quality of life due to comorbidities known as skeletal related events (SRE) (Orcajo-Rincon et al., 2022; Virk and Lieberman, 2007). These SREs - including severe pain, hypercalcemia, neurological deficits, pathological bone fractures, and spinal cord compression - lead to reduced activity and decreased quality of life (Mercadante, 1997; Zheng et al., 2022; So et al., 2012). Therefore, to immediately reduce the mortality and morbidity of cancer patients as well as improve patient well-being, treatment strategies to abate bone metastases are crucial.

Treatment strategies are relatively unique to bone metastases when compared to treatments for other metastases. In most cases of cancer metastasis, the treatment strategies for both the primary and secondary tumors are similar, where treatment methods function by either targeting tumors themselves or stimulating the immune system surrounding the tumors. For bone metastases, however, the treatment strategies focus on the function of the bone (an organ that continuously modifies itself throughout life by coupling osteoclast and osteoblast activity in a process called bone remodeling) (Hadjidakis and Androulakis, 2006). It has been established that the cells involved in bone remodeling (*e.g.*, osteoclasts, osteoblasts, and osteocytes) interact with metastatic cancer cells in the bone microenvironment. Here, the crosstalk between metastatic cells and bone-related cells act to further stimulate bone metastatic progression, known as "a vicious cycle of bone metastasis" (Guise, 2002; Cook et al., 2014). It is therefore logical for therapeutic strategies to target the interactions of bone remodeling to interfere with this vicious cycle.

In the clinic, drugs that decrease osteoclastic activity are often given as treatments to help combat the vicious cycle of bone metastases. The most commonly prescribed are bisphosphonates and denosumab, a human monoclonal anti-receptor activator of nuclear factor κB ligand (RANKL) antibody (Stopeck et al., 2010; Fizazi et al., 2011). These

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treatments have some success reducing some of the painful complications of bone metastases, but unfortunately, they ultimately fail to improve overall patient survival (Stopeck et al., 2010; Fizazi et al., 2011). In terms of targeting hydroxyapatite or osteoblastic bone metastatic lesions, clinical trials indicate that an alpha particle-emitting radiopharmaceutical, radium-223 dichloride ($^{223}RaCl_2$) can improve overall survival of prostate cancer patients with bone metastases but only by a few months (mean = 3 months) (Parker et al., 2013). Notably, to date, this is the only bone-targeted treatment option known to increase the survival time of patients with bone metastatic cancer. While several combinations of systemic treatments (*e.g.* hormone therapies, chemotherapies) are known to improve the overall survival of patients, they too only increase survival by a few months in patients with bone metastasis (McCain, 2014). Consequently, extirpating bone metastasis represents one of the greatest challenges of modern health care.

One of the reasons for challenges in developing treatments for bone metastasis is due in part to the intricate interactions between bone metastatic cancer cells and the bone marrow microenvironment, which contain a multitude of cell types, including cells of hematopoietic and mesenchymal origin, with diverging temporal dynamics (Kawai et al., 2018; Satcher et al., 2022). These interactions enable the vicious cycle of bone metastasis, as previously mentioned, where bone metastatic cancer cells stimulate osteolytic and/or osteoblastic activities of bone cells to provide space or growth factors for bone metastatic cancer cells to grow within the marrow (Guise, 2002). Additionally, the bone marrow microenvironment enhances bone metastatic progression through the high blood flow in the red marrow as well as the production of angiogenic factors, which provide adequate nutrient to support the survival of bone metastatic cancer cells (Suva et al., 2011). These add layers of complexity to understanding the mechanisms of bone metastasis creating a critical need to integrate our current understanding of cancer metastasis with emerging concepts in bone marrow microenvironment to advance our understanding of bone metastasis. Through this integration of knowledge, the overall goal is improving treatment strategies and clinical outcomes, while reducing the financial burden experienced by patients and their families.

Exosome-based cell-cell communication in the tumor microenvironment has recently been appreciated (Li et al., 2019). Exosomes are a type of extracellular vesicles surrounded by a lipid bilayer that carry proteins, DNA, RNA, and lipids that mediate cell communication (Gurunathan et al., 2019). They range in size from 40 to 120 nm in diameter and are derived from the endolysosomal pathway (El Andaloussi et al., 2013). Indeed, in the tumor microenvironment, exosomes derived from cancer-associated fibroblasts provide metabolites and tricarboxylic acid cycle (TCA) intermediates that can be utilized for metabolism to enhance cancer cell growth under nutrient stressed conditions (Zhao et al., 2016). Additionally, cancer-derived exosomes contribute to priming the secondary site for cancer cells to metastasize, referred to as "pre-metastatic niche formation" (Peinado et al., 2017; Yuan et al., 2021). It has been proposed that exosomes are also involved in the metastatic process of cancer cells by stimulating (i) extravasation of circulating tumor cells (Raskov et al., 2020) and (ii) colonization of metastatic cancer cells through fusion between cancer cells and cells in the metastatic sites (Hoshino et al., 2015). Particularly, prostate cancer-derived extracellular vesicles educate osteoblasts in order to create a favorable environment for further dissemination of prostate cancer to the bone (Furesi et al., 2021). Furthermore, microRNA (miRNA) in cancer-derived exosomes is known to induce osteoblastic differentiation, resulting in the release of growth factors, which initiate the vicious cycle and subsequently enhance the bone metastatic progression (Furesi et al., 2021; Masaoutis and Theocharis, 2019) (Table 1). These findings suggest the exosomes serve as one of the key regulators in the development of bone metastasis.

While the impact of exosomes on the development of bone metastasis has recently been revealed, complete mechanisms whereby exosomes contribute to the formation of the metastatic niche in the bone and how different exosomal-cargo (miRNA, metabolites) impacts bone-specific metastasis still remain to be elucidated. In this review, we therefore will (i) describe the roles of cancer-derived exosomes in bone metastatic progression by focusing on the cancer/bone marrow microenvironment interactions, (ii) introduce the concept that exosomes are involved in the development of SRE by paying special attention to cancer-induced bone pain, and (iii) discuss the potential impacts of exosome functions on the development of therapies and diagnostic tools for bone metastasis.

2. The roles of exosomes in cancer dissemination to bone and colonization in bone

The beginning of the exosome journey starts within cells. Initially, a portion of plasma membrane invaginates carrying cargo into the

Table 1
Exosomal miRNAs and their potential effects on bone metastasis.

Exosomal miRNA	Cancer type	Cell of origin	Recipient cell	Effect	Reference
miR-19a	Breast	Cancer cells	Osteoclasts	Promotes osteoclastogenesis	(Wu et al., 2021)
miR-21	Breast, Lung	Cancer cells	Osteoclasts	Promotes osteoclast differentiation	(Yuan et al., 2021) (Xu et al., 2018)
miR-23b	Breast	Bone marrow-derived mesenchymal stem cells	Cancer cells	Induces tumor dormancy	(Ono et al., 2014)
miR-23c	Prostate	Cancer cells	Human umbilical vein endothelial cells	Induces tumor dormancy dormancy by suppressing CXCL12 expression in human umbilical vein endothelial cells	(Sharma et al., 2021)
miR-26a-5p, miR-27a-3p, and miR-30e-5p	Prostate	Cancer cells	Osteoblasts	Inhibits osteoblast differentiation	(Furesi et al., 2022)
miR-92a-1-5p	Prostate	Cancer cells	Osteoclasts, Osteoblasts	Promotes osteoclast differentiation, inhibits osteoblast differentiation	(Yu et al., 2021)
miR-141-3p	Prostate	Cancer cells	Osteoblasts	Enhances osteoblastic metastatic lesions	(Ye et al., 2017)
miR-192	Lung	Cancer cells	Human umbilical vein endothelial cells	Inhibits osteoclastogenesis by impairing tumor-induced angiogenesis	(Valencia et al., 2014)
miR-214-3p	Breast	Cancer cells	Osteoclasts	Promotes osteoclast differentiation	(Liu et al., 2017)
miR-222/223	Breast	Bone marrow-derived mesenchymal stem cells	Cancer cells	Induces tumor dormancy	(Bliss et al., 2016)
miR-503-3p	Breast	Peripheral blood mononuclear cells	Osteoclasts	Inhibits osteoclast differentiation	(Chen et al., 2014; Zhao et al., 2017)
miR-940	Breast	Cancer cells	Bone marrow-derived mesenchymal stem cells	Promotes osteogenic differentiation of bone marrow-derived mesenchymal stem cells	(Hashimoto et al., 2018)
miR-1273 g-3p	Breast	Cancer cells	Bone marrow-derived mesenchymal stem cells	Promotes osteogenic differentiation of bone marrow-derived mesenchymal stem cells	(Sun et al., 2022)

cytoplasm and traffics to the early endosome, where the invaginated plasma membrane's cargo is sorted (Gurung et al., 2021). Thereafter, the early endosome matures into a multivesicular body, or late endosome, through inward budding of its membrane, resulting in the formation of intraluminal vesicles (Zhang et al., 2019b; Alenquer and Amorim, 2015). During this maturation process within the multivesicular body, intraluminal vesicles are known to obtain several cargos (Gurung et al., 2021). Eventually, these multivesicular bodies fuse with the plasma membrane, leading to the secretion of their intraluminal vesicles, which are known as extracellular vesicles, or exosomes, once they are released from the cell (Théry et al., 2009; Zhang et al., 2019b). After being released into the extracellular space, exosomes and their cargo can reach cells in proximal sites by direct interaction, as well as cells in distant sites through circulation (Głuszko et al., 2019). The nature of the encapsulated cargos carried by exosomes is thought to be strongly influenced by the parental cell. Indeed, it has been demonstrated that exosomal miRNA profiles reflect their cells of origin (Corcoran et al., 2014; Wang and Gires, 2019).

The way in which exosomal contents are taken up by target cells is through either (i) direct fusion with the membrane; (ii) receptor-ligand interactions; or (iii) endocytosis (Zhang et al., 2019b). Furthermore, this uptake of exosomal cargo can transfer phenotypic traits of parental cells to recipient cells (Corcoran et al., 2014; Wang and Gires, 2019). For example, normal human bronchial epithelial cells (HBECs) acquired resistance to the chemotherapeutic agent gemcitabine *in vitro*, when they were exposed to exosomes derived from HBECs that were transformed by introducing three common non-small cell lung cancer oncogenic mutations (Lobb et al., 2017). Additionally, exosomes can change the phenotype of recipient cells with their cargo. It has been demonstrated that macrophage migration inhibitory factor within pancreatic ductal adenocarcinomas-derived exosomes induced pre-metastatic niche formation in the liver by (i) inducing transforming growth factor β (TGF-β) production in Kupffer cells, (ii) up-regulating fibronectin expression in hepatic cells, and (iii) recruiting bone marrow-derived macrophages into the liver (Costa-Silva et al., 2015). While this finding suggests that exosomes can be involved in the dissemination process of cancer by influencing the cells in the secondary sites, less is known as to how exosomes contribute to bone metastatic formation.

It has been suggested that bone metastatic cancer cells home to the bone marrow through the C-X-C Motif Chemokine Receptor 4 (CXCR4)/C-X-C Motif Chemokine Ligand 12 (CXCL12) axis (Taichman et al., 2002; Müller et al., 2001). Briefly, CXCR4 (a receptor for CXCL12)-expressing bone metastatic cancer cells migrate towards CXCL12 expressed by cells in the bone marrow (*e.g.* endothelial cells, stromal cells, osteoblasts) (Wang et al., 2006). Similar to the pre-metastatic niche formation in pancreatic cancer liver metastasis, exosomes also contribute to the priming of the bone marrow microenvironment. In a rodent model, prostate cancer-derived exosomes obtained from prostate cancer patients' serum up-regulated CXCL12 expression in the bone marrow stromal cells by transferring pyruvate kinase M2, resulting in (i) the higher incidence of bone metastasis than lung and kidney metastases and (ii) subsequent bone metastatic progression (Dai et al., 2019). Additionally, an *in vitro* study demonstrated that osteotropic human melanoma LCP-Mel-derived exosomes reprogramed non-osteotropic human melanoma cells (SK-MEL28, WM-266) to acquire the osteotropic ability towards CXCL12-secreting bone fragments by up-regulating their CXCR4 expression (Mannavola et al., 2019). Moreover, miR-192-enriched exosomes derived from human lung cancer A549 cells impaired their bone colonization by preventing angiogenesis in the marrow through suppression of the pro-angiogenic factors CXCL1 and interleukin (IL)-8 (Valencia et al., 2014). These findings suggest that bone metastatic cancer cell-derived exosomes control dissemination of cancer cells to bone and their colonization in bone.

3. The impacts of exosomes on the phenotype of bone metastatic lesions

Under normal physiological conditions, the bone resorption mediated by osteoclasts and the bone formation mediated by osteoblasts are balanced (Li and Wang, 2021), leading to proper bone remodeling. However, when cancer metastasizes to the bone, this well-balanced homeostasis of the bone microenvironment loses this equilibrium becoming dysregulated, resulting in bone metastatic growth. The phenotype of bone metastatic lesions is determined by how bone metastatic cancer cells activate cells involved in bone remodeling, including osteoclasts and osteoblasts. When bone metastatic cancer cells dominantly stimulate the activities of osteoclasts, the lesions of bone metastases become osteolytic, whereas those of bone metastases result in osteogenesis when bone metastatic cancer cells interact more with osteoblasts.

As stated above, the interaction among the bone metastatic cancer cells and cells involved in the bone remodeling is called a vicious cycle of bone metastasis. It has been suggested that this vicious cycle is controlled by several growth factors and/or cytokines [*e.g.* bone matrix (degraded by osteoclasts)-derived transforming growth factor beta (TGF-β) and endothelin-1 (Furesi et al., 2021; Esposito et al., 2018); osteoblasts-derived RANKL (Esposito et al., 2018); and bone metastatic cancer cells-derived parathyroid hormone-related protein (PTHrP) (Guise et al., 1996)]. However, recent work has demonstrated that exosome-derived bone metastatic cancer cells add another level of complexity in the vicious cycle of bone metastasis by affecting the function and activity of bone-related cells.

Indeed, prostate cancer-derived exosomes induced survival, proliferation, and mineralization of murine pre-osteoblastic cell line MC3T3-E1 cells through the mitogen activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) pathway *in vitro* (Borel et al., 2020; Karagiosis et al., 2009). Along with this concept, in a rodent model, greater osteoblastic bone metastatic burden and lower median survival of the animals inoculated with prostate cancer-derived exosomes over-expressed miR-141-3p were observed, compared to those of animals inoculated with miR-141-3p intact prostate cancer-derived exosomes (Ye et al., 2017). In another rodent study, breast cancer-derived exosomes (which contains long noncoding RNA SNHG3) induced osteolytic bone metastatic lesions by down-regulating exosomal miR-1273 g-3p (Sun et al., 2022). This osteolytic bone metastasis was reversed by differentiating bone marrow stromal cells into osteoblasts through the upregulation of exosomal miR-1273 g-3p when SNHG3 was down-regulated in breast cancer (Sun et al., 2022). Interestingly, when a triple negative breast cancer cell line MDA-MB-231 cells, known to develop osteolytic bone metastatic lesions, were transfected with hsa-miR-940 (known to induce osteoblastic differentiation), exosomal hsa-miR-940 induced the development of osteoblastic bone metastatic lesions in the hsa-miR-940-over-expressing MDA-MB-231 cells-bearing animals (Hashimoto et al., 2018).

Alternatively, exosomes have been shown to enhance or reduce osteoclast activity. Non-small cell lung cancer-derived exosomes that carry amphiregulin (a ligand for epidermal growth factor receptor, EGFR) induce the differentiation of pre-osteoclasts to osteoclasts through EGFR expressed by pre-osteoclasts (Taverna et al., 2017). Metastatic breast cancer cells-derived exosomal miR-21 significantly increased bone metastatic burden at the hind-limbs of cancer-bearing rodents by enhancing osteoclastogenesis through downregulation of programmed cell death 4 (PDCD4), known to inhibit osteoclast differentiation and functions (Yuan et al., 2021). Similarly, exosomal miR-21 derived from human lung adenocarcinoma cell line A549 cells promoted osteoclastic differentiation of murine primary bone marrow monocytes into tartrate-resistant acid phosphatase (TRAP, known to reflect osteoclastic differentiation) positive osteoclasts, by downregulating PDCD4 (Xu et al., 2018). In a rodent study, estrogen receptor (ER) positive breast cancer cells expressing integrin-binding sialoprotein (IBSP)

recruited osteoclast precursors to the bone marrow (Wu et al., 2021). In turn, these osteoclast precursors activated nuclear factor kappa B (NF-κB) and protein kinase B (AKT) pathways due to the suppression of PTEN expression by exosomal miR-19a-derived from metastatic breast cancer, which ultimately resulted in bone metastatic progression (Wu et al., 2021). Moreover, MDA-MB-231 cells-derived exosomal miR-214-3p induced increased serum bone resorption marker, type I collagen cross-linked C-telopeptide (CTX-1) levels and increased osteoclast numbers in the bones in the MDA-MB-231-bearing mice, by directly downregulating TNF receptor-associated factor 3 [TRAF3, known to limit RNKL-inducing osteoclastogenesis (Boyce et al., 2018)] levels in osteoclats (Liu et al., 2017).

Intriguingly, exosomal miR-92a-1-5p derived from the human prostate cancer cell line MDA PCa 2b cells, known to induce osteoblastic bone metastatic lesions, increased mRNA expression of the osteoclast markers, cathepsin K (CTSK) and RANK, and TRAP activity in murine monocyte/macrophage cell line RAW 264.7 cells *in vitro* (Yu et al., 2021). As expected, this osteoclastic differentiation mediated by exosomal miR-92a-1-5p was reversed with anti-miR-92a-1-5p (Yu et al., 2021). Exosomal miR-92a-1-5p also decreased a mineralization marker Alizarin Red S in MC3T3-E1 cells *in vitro* and degraded bone extracellular matrix of MDA PCa 2b-baring mice by down-regulation on of collagen type I alpha 1 (COL1A1, type I collagen subunit which is a major component of bone extracellular matrix) *in vivo* (Yu et al., 2021). Similarly, exosomes derived from murine prostate cancer cell line RM1-BM cells, diminished expression of osteoblastic differentiation markers, bone morphogenetic protein 2 (BMP2) and RUNX family transcription factor 2 (RUNX2) in osteoprogenitor cells *in vitro* by releasing miR-26a-5p, miR-27a-3p, and miR-30e-5p (Furesi et al., 2022). Conversely, another study demonstrated that prostate cancer-derived exosomes decreased the expression of differentiation markers of monocytic osteoclast precursors (Karlsson et al., 2016).

Collectively, these findings suggest that cancer-derived exosomes are involved in the determination of the phenotype of the bone metastatic lesions, although how exosomal miRNA influence on the phenotype of the bone metastatic lesions are still controversial.

4. Contribution of exosomes to the regulation of tumor cell dormancy in the bone

One of the major challenges in the treatment of bone metastasis is the development of tumor dormancy in the bone. It has been demonstrated that bone metastatic cancer cells utilize the bone marrow microenvironment to acquire a dormant state (Shiozawa et al., 2010). Bone metastatic tumor dormancy is defined as a lag occurring between early cancer bone colonization and full-blown bone metastatic progression (Senft and Ronai, 2016). During this period, bone metastatic cancer cells are thought to arrest in a quiescent-like state, resulting in escaping from the cytotoxic treatments, including chemotherapy and radiation, that mainly target proliferating cells (Attaran and Bissell, 2022). These escaped calls remain undetectable for years or decades, and then eventually re-grow and become incurable.

Recent studies suggest that exosomes are involved in the regulation of tumor dormancy and have a mixed role in this process by promoting or suppressing quiescence. For example, while exosomes derived from bone marrow-derived mesenchymal stem cells induced cell cycle arrest in breast cancer cells (Bliss et al., 2016), bone marrow stromal cell-derived M1 macrophage-secreted and cancer associated fibroblasts-derived exosomes helped breast cancer cells exit from a dormant state (Walker et al., 2019; Sansone et al., 2017). In the contexts of bone metastasis, regucalcin (RGN)-overexpressing human prostate cancer cell line C4-2B and PC-3 cells showed smaller bone metastatic burden than that mediated by control cells through tumor dormancy *in vivo* (Sharma et al., 2021). This tumor dormancy was directly induced by prostate cancer cell-derived exosomes carrying miR-23c and indirectly by miR-23c-induced neoangiogensis suppression (Sharma et al., 2021). On the other hand, exosomes-derived bone marrow mesenchymal stem cells, carrying miR-23b induced dormancy of bone tropic MDA-MB-231 cells (BM2 cells), by downregulating myristoylated alanine rich C-kinese substrate (MARCKS, known to promote cell cycling and motility, in the bone of BM2-bearing mice (Ono et al., 2014). Although the roles of exosomes have been appreciated, further studies are clearly warranted to more detailed and precise mechanisms underlying the regulation of tumor dormancy in the bone by exosomes.

5. A potential roles of exosomes in the development of cancer-induced bone pain

Exosomes may not only be involved in the establishment of cancer bone metastasis but also regulate the development of its associated complications, especially cancer-induced bone pain. An estimated 60–84 % of cancer patients with bone metastasis experience a varying degree of bone pain (Zajączkowska et al., 2019), with up to 50 % of them describing the management of pain by present treatments as poor (Mantyh, 2014). Therefore, understanding the molecular underpinnings of cancer-induced bone pain would be of great value to develop therapeutics that are more effective and safer; subsequently, patients' quality of life can be improved. One proposed mechanism of the induction of cancer-induced bone pain is through the tumor microenvironment's harmful stimuli that causes excitation, sensitization, and electrophysiological changes of peripheral primary sensory nerves (Falk and Dickenson, 2014; Mantyh, 2014; Ivanusic, 2017; Yoneda et al., 2021). Interestingly, recent studies demonstrated that cancer-derived exosomes are also involved in this nociception process.

Indeed, a recent study demonstrated that the human lung cancer cell line A549-derived exosomal miRNA, let-7d-5p, induced cancer-induced bone pain by inhibiting the μ-opioid receptor 1 expression in dorsal root ganglia neurons, and the let-7d-5p antagomir inhibited spontaneous flinching of mice inoculated with A549 cells directly into their femur (Li et al., 2021). Additionally, it has been demonstrated that M2 macrophage-derived extracellular vesicles, carrying miR-216A, enhanced antinociception in the mice inoculated with rat breast cancer cell line Walker 256 cells directly into their femur by suppressing high mobility group box 1 (HMGB1) expression, known to be involved in the nociception process in the chronic pains (Klune et al., 2008; Liu et al., 2020) and the toll-like receptor 4 (TLR4)/NF-κB pathway, known to regulate the expression of a variety of inflammatory cytokine genes (Cai et al., 2021; Kawai and Akira, 2007), in their spinal cord (Cai et al., 2021). Although more comprehensive studies are necessary to fully elucidate the roles of exosomes in cancer-induced bone pain, modulation of proteins in the tumor microenvironment through exosomal-derived cargos can be a new therapeutic strategy to ameliorate cancer-induced bone pain.

6. Exosomes can be used as new theranostic biomaterials for bone metastasis

Since, as discussed above, recent evidence suggest that exosomes are involved in several aspects of the bone metastatic process, exosomes can be used for both treatment and diagnostic options for bone metastasis. Exosomes can (i) influence both cancer cells and cells in the bone microenvironment by delivering cargos and (ii) travel stably throughout the body. Therefore, potential theranostic applications using cancer-derived exosomes for bone metastasis are as follows: (i) a treatment target (Datta et al., 2017); (ii) a treatment derivative (Haney et al., 2020); and (iii) a circulating biomarker (Thakur et al., 2014) (Fig. 1).

6.1. *Targeting exosome biogenesis and secretion*

Because of the recent findings of exosomal roles in cancer development, targeting exosome biogenesis, secretion, and fusion have been proposed as ways to mitigate cancer progression and metastasis. Indeed,

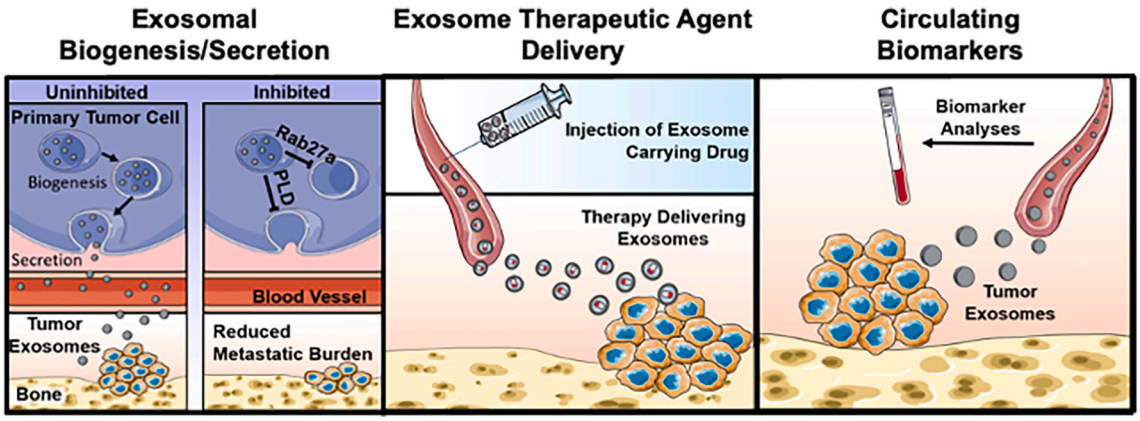

Fig. 1. Exosomes as theranostic options for bone metastasis.
Tumor exosomes derived from primary tumor cells through biogenesis and secretion are thought to contribute to bone metastatic progression in the marrow by traveling through circulation to the bone marrow microenvironment. Due to their roles in enhancing the bone metastatic process, exosomes may also prove to be a useful therapeutic target. As shown in the left panel, targeting exosomal biogenesis through inhibitors such as Ras-related protein (Rab27a) and exosomal secretion *via* inhibitors such as phospholipase D (PLD) may help inhibit tumor exosomes from carrying out their traditional role as a metastatic catalyst, resulting in a reduced metastatic burden. In the middle panel, the potential use of exosomes as drug delivery vehicles as a new treatment strategy for bone metastasis are described. Since systemic delivery of exosomes originating from cells in the bone marrow has been known to have a homing ability to the bone and cytotoxic agents can be loaded into exosomes, cytotoxic agent-loaded exosomes derived from bone marrow cells can be utilized to specifically target bone metastasis. Finally, the right panel illustrates the potential use of exosomes as circulating biomarkers or a liquid biopsy. Although a specific exosomal biomarker for bone metastasis has not yet been identified, the role of exosomes as biomarkers of other bone-centric diseases has already been demonstrated. Therefore, exosomes may aid in the diagnosis of bone metastasis as bone metastasis also results in bone destruction and an imbalance in osteoblast/osteoclast activity. Graphics adapted from Smart Servier Medical Art (https://smart.servier.com/).

in a melanoma mouse study, intratumoral injection of GW4869, an inhibitor of exosome secretion, significantly reduced melanoma (B16-BL6 cells) tumor growth (Matsumoto et al., 2017). Additionally, downregulation of Ras-related protein Rab27A, known to regulate exosome biogenesis (Ostrowski et al., 2010), in the murine melanoma cell lines B16-F10 and SK-Mel-2 resulted in (i) a 50 % reduction in exosome secretion *in vitro*, and (ii) suppression of primary tumor growth and lung metastasis *in vivo* (Peinado et al., 2012). Moreover, a natural microbial metabolite, Manumycin-A, inhibited exosome biogenesis and secretion in the human castration-resistant prostate cancer cell line C4-2B cells by downregulating the Ras/Raf/ERK1/2 signaling pathway *in vitro* (Datta et al., 2017). Similarly, an inhibitor for a mediator of membrane fusion and exosome secretion, phospholipase D (PLD), decreased exosome secretion and downstream alkaline phosphatase (osteogenic differentiation marker) expression of C4-2B cells (Borel et al., 2020), suggesting that targeting and blocking the generation and release of exosomes, that are associated with contribution to metastasis, may a therapeutic strategy for precluding bone metastasis. More studies including *in vivo* as well as clinical experiments are clearly warranted to further demonstrate this notion.

6.2. Delivering therapeutic agents to bone marrow as a vehicle

Nanoparticles have been used as a drug delivery vehicle, and the drug delivery systems with nanoparticles have been somewhat successful. However, there have still been concerns about its heightened immune response, associated toxicity, and/or lack of therapeutic efficacy (Zolnik et al., 2010). Recent pre-clinical studies have revealed that exosomes can be an alternative drug delivery vehicle due in part to its ability to evade immune clearance (Kalluri and LeBleu, 2020). Interestingly, a more recent study demonstrated that the exosome delivery method can enhance treatment efficacy of chemotherapeutic agents, compared to the conventional delivery methods. In this study, murine triple negative breast cancer cell line T11 cells and human triple negative breast cancer cell line MDA-MB-231 cells were orthotopically inoculated into immunocompetent and immunocompromised mice, respectively, and these mice were treated with either chemotherapeutic agent (paclitaxel for MDA-MB-231 cells and doxycycline for T11 cells) or exosomes loaded with corresponding chemotherapeutic agent (Haney et al., 2020). The sizes of the tumor treated with chemotherapeutic agent-loaded exosomes were significantly smaller than those treated with chemotherapeutic agent (Haney et al., 2020), suggesting that exosomes, which are derived from the cells that have tumor homing ability, can target cancer specifically even with systemic delivery. This results in enhancing efficacy and minimizing adverse effects of cytotoxic agents. Moreover, exosomes derived from bone marrow mesenchymal stem cells have an ability to preferentially homing to bone (Hu et al., 2021; Naseri et al., 2018). Taken together, these findings suggest that exosomes derived from bone marrow cells can be used to deliver cytotoxic agents straight to the bone that is notoriously known to be a hard-to-reach organ.

6.3. Serving as a circulating biomarkers

Another possible theranostic application of exosomes is through their use as a circulating biomarker (Thakur et al., 2014). The abundance of exosomes and their cargo present in most bodily fluids (*e.g.*, plasma/serum, saliva, urine, fecal matter, *etc.*) makes them a compelling clinical tool due to the ease of sample collection (Barile and Vassalli, 2017; Zhang et al., 2019a). To date, detecting bone metastasis in its initial stages is extremely difficult. The first sign of bone metastasis in patients is often progressively worsening pain or other SREs, and imaging is then needed to confirm the presence of bone metastatic disease (Jayarangaiah et al., 2022). Since metastatic lesions are detected everywhere in the body when diagnosed and are thus difficult to treat, treatment options left for the patients with bone metastases are primarily palliative. Therefore, early detection of this fatal metastatic disease is much needed. Although plasma exosomal miRNA have been identified as a circulating biomarker for castration resistant prostate cancer (prostate cancer patients with castration resistant prostate cancer likely develop bone metastasis) (Huang et al., 2015), a specific exosomal biomarker for bone metastasis has not been identified yet.

However, exosomes have proven to serve as biomarkers in other bone-centric diseases, although these diagnostic measures have not yet been implemented clinically. In the serum of patients with osteoporosis, exosomal circular RNA Has_circ_0006859 was upregulated and thought

to function as a biomarker (Zhi et al., 2021). This circular RNA, through its role as a miRNA sponge, induces osteoporosis by promoting the differentiation of bone marrow mesenchymal stem cells into adipocytes and inhibit the differentiation of osteoblasts through the down-regulation rho associated coiled-coil containing protein kinase 1 (ROCK1) following the upregulation of miR-431-5p (Zhi et al., 2021). Further, exosomal miR-324-3p, miR-766-3p, miR1247-5p, miR-330-5p, and miR-3124-5p were all found to be differentially expressed in patients with osteoporosis ($n = 16$) when compared with a control group ($n = 18$) suggesting their potential role as biomarkers for this bone degenerative disease (Shi et al., 2022). Similarly, significantly lower exosomal miR-193b-3p, known to regulate chondrocyte formation and metabolism, was found in the plasma of patients with osteoarthritis, compared to that of healthy controls, highlighting its role as a potential diagnostic marker (Meng et al., 2018). Seeing as bone metastasis also results in bone destruction and an imbalance in osteoblast/osteoclast activity, these early exosomal diagnostic techniques used for other bone-tropic diseases (osteoarthritis and osteoporosis) can be useful in the early detection of bone metastasis and may offer opportunity to treat the disease before many of the complications can occur.

7. Conclusion and future perspectives

Our understanding of exosomes and their role in cancer has significantly developed over the past decades. While exosomes were originally thought to be essentially part of a garbage disposal system ridding the cell of waste (H Rashed et al., 2017), recently studies have demonstrated that exosomes play an important role in cancer progression, including the bone metastatic process. As discussed above, exosomes are involved in (i) the induction of bone tropism in the cancer cells; (ii) the establishment of favorable environment for bone metastatic cancer cells; (iii) the growth of bone metastatic cancer cells; and (iv) the development of complications of bone metastasis (Fig. 2). Currently, when patients are diagnosed with bone metastasis, they are generally considered incurable (Weilbaecher et al., 2011), and the level of complexity of the bone microenvironment proves to be responsible for this clinical obstacle. Since exosomes have begun to be known as part of the regulation of the complex intercellular communication between bone metastatic cancer cells, the cells of hematopoietic origin (Niazi et al., 2020), and the cells of mesenchymal origin (Vakhshiteh et al., 2019), the understating of complete mechanisms whereby exosomes controls these cellular cross-talks may provide new insights into the pathophysiology and treatment of bone metastatic disease.

Despite notable scientific advancements in the early diagnosis and treatment of primary tumors, there is ultimately no curative treatment for bone metastatic disease. Following diagnosis with bone metastasis, patients and their families are subject to enormous financial, emotion, and physical stress as a result of the disease. Financial costs associated with bone metastasis proves to be problematic for the health care system as well as patients with bone metastasis have become increasingly expensive to care for in recent years. As previously mentioned, the current standards of care for treating bone metastasis includes using anti-resorptive bone-targeting agents (*e.g.* bisphosphonate, denosumab) and/or external beam radiotherapy. While the alpha-emitting pharmaceutical $^{223}RaCl_2$ can extend overall survival in bone metastatic prostate cancer patients, it is only by a few brief months. Unfortunately, the clinical benefits provided to patients by current therapies are modest. Furthermore, these treatments mainly target bone remodeling.

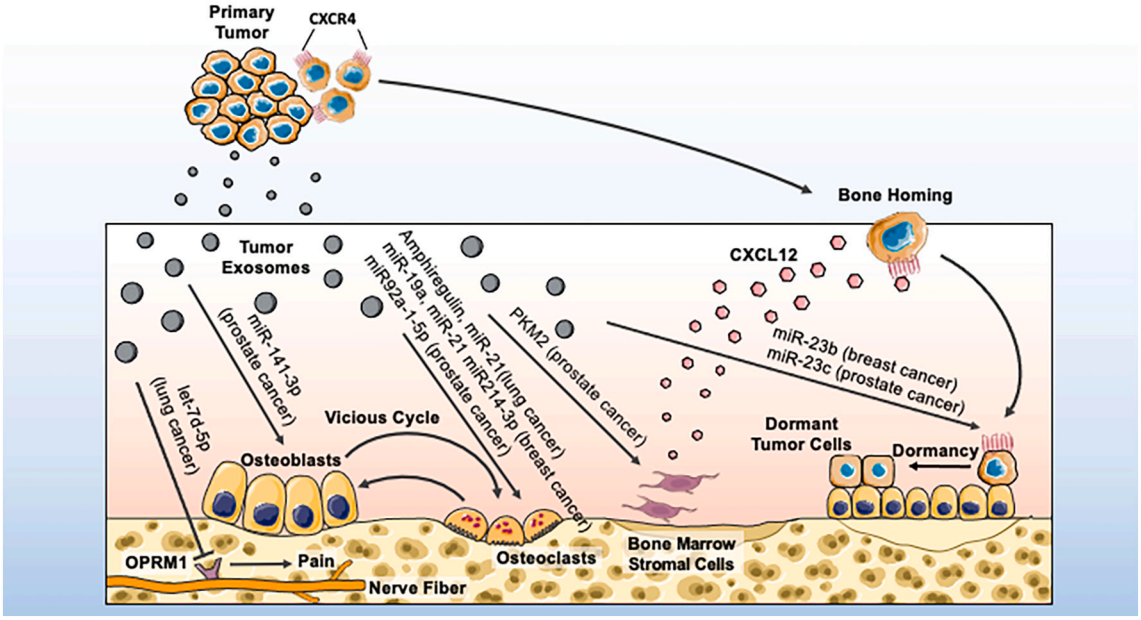

Fig. 2. The Proposed mechanisms where by exosomes contribute to bone metastatic progression.
Exosomes derived primary tumor induce bone tropism in the cancer cells by over-expressing C-X-C Motif Chemokine Receptor 4 (CXCR4), a chemokine receptor known to be associated with cell migration; and these tumor exosomes upregulate of C-X-C Motif Chemokine Ligand 12 (CXCL12) in bone marrow stromal cells through delivering pyruvate kinase M2 (PKM2) to bone marrow stromal cells (observed in prostate cancer). CXCR4-expressing cancer cells are known to migrate towards CXCL12 in the bone marrow as an osteotropic axis, resulting in higher incidence of bone metastasis. Following cancer cell homing to the bone, tumor dormancy is further promoted by exosomes carrying miR-23b derived from bone marrow mesenchymal stem cells (observed in breast cancer) or miR-23c derived from prostate cancer cells (observed in prostate cancer). Exosomal cargo also interacts directly with cells in the bone to create an environment favorable for metastasis. Exosomes carrying miR-141-3p act on osteoblasts contributing to greater osteogenesis and metastatic burden (observed in prostate cancer). Further, amphiregulin (lung cancer), miR-21 (lung cancer), miR-19-a (breast cancer), miR214-3p (breast cancer), or miR92a-1-5p (prostate cancer) from cancer-derived exosomes may act on osteoclasts by inducing their differentiation from pre-osteoclasts to osteoclasts. Following transport to the bone marrow microenvironment, exosomes have also been shown to promote cancer-induced bone pain through release of let-7d-5p, which inhibits the mu (μ) opioid receptor (OPRM1) in sensory nerves (observed in lung cancer). Graphics adapted from Smart Servier Medical Art (https://smart.servier.com/ (web archive link,)).

Therefore, new approaches that target factors other than bone remodeling are needed if eradicating bone metastasis is to be an achievable clinical goal. One of the difficulties in treating bone metastasis is that bone metastatic cancer cells acquire dormancy in the marrow. Since exosomes can transfer their cargo to the other cells, they can be used as a new therapeutic option to awake dormant bone metastatic cancer cells by delivering cargos that can reverse cell cycle arrest so that conventional cytotoxic agents, which target rapidly dividing cells, can attenuate these awakened bone metastatic cancer cells. Although further studies are undoubtedly necessary based on evidence supporting the roles of exosomes in bone metastatic development and progression as well as its resultant painful complications, translating therapies targeting and/or utilizing exosomes from bench to bedside may give rise to desperately needed palliative and curative treatment options for cancer patients with bone metastasis.

CRediT authorship contribution statement

J.O., K.F.C., G.D., and Y.S. wrote the manuscript. All authors reviewed and approved the final manuscript.

Declaration of competing interest

Yusuke Shiozawa has received research funding from TEVA Pharmaceuticals, but not relevant to this study. No conflict of interest exists for remaining authors.

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