

Cancer metastasis to the bone: Mechanisms and animal models (Review)

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Abstract. The majority of cancer-related deaths result from tumor metastasis, with bone metastasis occurring in almost all types of malignant tumors. Understanding the mechanism by which tumors metastasize to bone is critical for the identification of novel therapeutic targets. A large amount of research has been carried out using animal models, and these models have been crucial in advancing the fundamental understanding of cancer. However, current models are limited; although they can mimic specific stages of the metastatic process, they are not able to replicate the entire process from tumorigenesis to bone metastasis. The present review describes the molecular changes that occur in the intraosseous microenvironment of bone metastases, including osteolytic and osteoblastic types, and summarizes advancements in animal models of bone metastasis.

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1. Introduction

Cancerous tumors are a leading cause of death worldwide (1). Tumors are classified as primary and secondary. Primary tumors originate from normal cells that undergo malignant transformation due to various internal and external carcinogenic factors and form a cancer cell mass (2). Malignant cells can leave the primary tumor and spread to other parts or organs of the body via complex mechanisms, resulting in secondary or metastatic cancers (2). Bone is one of the most common sites of metastasis, with bone metastasis affecting >1.5 million patients worldwide (3). Bone metastasis frequently occurs in numerous cancers (4), particularly prostate cancer, breast cancer and lung cancer (1,5). Metastasis is often considered as the terminal stage in the progression of cancer, and tumor cells in the bone microenvironment have been suggested to undergo reprogramming, enabling them to seed secondary metastasis to other organs including the lung, liver, and brain (6). Once bone metastases occur, they are incurable and lead to severe skeletal-related events, including pathological fractures, pain, compression of the spinal cord or nerves, and disability in patients with advanced bone metastases (5). These events critically reduce the quality of life of patients with bone metastases and increase medical costs (7). Therefore, it is urgently necessary to elucidate the mechanisms of bone metastasis, develop animal models to better understand the characteristics of bone metastasis, and identify new therapeutic targets for bone metastasis. The present review focuses on the mechanisms underlying bone metastasis and recent progress in animal models of bone metastasis.

2. Dynamic cycle of bone remodeling

Bone undergoes dynamic remodeling throughout life (8) (Fig. 1). This process promotes bone regeneration and repair, which are crucial for the maintenance of bone homeostasis. Dynamic bone remodeling primarily involves osteolysis and bone formation.

Dissolution of bone. The dynamic bone remodeling cycle begins when osteoclast precursors are attracted to sites of bone damage or aging in response to chemokine signaling (9). These

precursors then differentiate and fuse to form multinucleated osteoclasts, which play an osteolytic role. The binding of receptor activator of nuclear factor- κ B (RANK) with RANKL (RANKL) promotes the fusion, differentiation and maturation of osteoclast precursors (10). RANK is expressed on the cell surface of osteoclasts and their precursors, while RANKL is produced primarily by osteocytes, osteoblasts, bone marrow stromal cells and activated T cells (11). Osteoprotegerin (OPG) also binds to RANKL, which prevents RANKL from binding to RANK, thereby blocking RANKL signaling and regulating osteoclastogenesis and osteolysis (12). In addition, Nozawa *et al* (13) demonstrated that cellular communication network factor 2 (CCN2), also known as a connective tissue growth factor, induces osteoclast formation through interaction with integrin α v β 3. CCN2 also regulates dynamic bone remodeling by participation in the RANK-RANKL-OPG system (14). It has been suggested that bone morphogenetic protein 9 (BMP9) can inhibit bone metastasis in patients with breast cancer by downregulating CCN2 (15). Following osteoclast differentiation and maturation, cytoskeletal changes lead to the formation of a ruffled border. The osteoclasts then secrete proteolytic enzymes and hydrochloric acid through the ruffled border to facilitate osteolysis (15).

Formation of bone. Following osteolysis, osteoclasts leave the bone surface and undergo programmed cell death, specifically apoptosis, which signals the beginning of bone formation (16). Osteoblast precursors are attracted to the site of osteolysis. This process is regulated by the osteoblast precursor transcription factors, including core-binding factor subunit a-1, also known as Runt-related transcription factor 2 (RUNX2), and osterix, which bind to osteoblast-specific gene enhancers to promote the development of an osteoblast-like phenotype (17). In addition, BMPs promote the proliferation and differentiation of osteoblast precursors (18), and Wnt family proteins promote bone formation via the activation of low-density lipoprotein receptor-related protein 5 (LRP5), RUNX2 and osterix (19). However, sclerostin (Sost) produced by osteocytes inhibits bone formation by antagonizing the effects of Wnt proteins (20). Mature osteoblasts secrete noncalcified bone matrix onto the bone surface, which subsequently mineralizes to form mature bone. During this process, some osteoblasts are trapped by mineralized bone and differentiate into osteocytes. Osteocytes are linked together by elongated cytoplasmic extensions, which allows them to transmit signals, such as mechanical loading signals, via nitric oxide and prostaglandin signaling molecules (21).

Dynamic bone remodeling continues when bone formation is complete. Various factors influence this dynamic process, which can be classified into two categories: i) Chemical factors or hormones and ii) physical factors (22). Inflammatory factors such as IL-1 and tumor necrosis factor accelerate the remodeling cycle. Hormones that regulate calcium levels, such as 1,25-dihydroxyvitamin D and parathyroid hormone, promote bone remodeling and mobilize bone calcium to maintain blood calcium levels (23). While thyroid and growth hormones promote bone remodeling (24), estrogens and androgens inhibit bone resorption and promote bone formation, particularly in the trabecular and endocortical skeletal compartments (25). These factors regulate bone remodeling primarily by modulating

the RANK-RANKL-OPG signaling pathway (26). Notably, mechanical loading affects bone remodeling; for example, increased mechanical loading increases bone formation and reduces osteolysis.

3. Physiological changes during cancer bone metastasis

Bone metastasis has two main phenotypes: Osteolytic and osteogenic. Most bone metastases exhibit both osteolytic and osteogenic characteristics, with one phenotype being dominant. For example, breast and lung cancers are frequently osteolytic, while prostate cancer is frequently osteoblastic. Osteolytic lesions are distinguished primarily by bone destruction, which generally appears as cortical cavitation when analyzed using radiographic imaging. By contrast, osteoblastic lesions are distinguished by the excessive formation of new bone, which results in increased bone density on imaging, frequently described as osteosclerosis on the bone surface (2).

Mechanisms of osteolytic metastasis

Tumor osteolytic microenvironment. Bone remodeling involves a variety of cytokines, growth factors and cell adhesion molecules, which makes bone an attractive location for metastatic tumor cells (Fig. 2). The epiphysis, with its rich blood supply and trabecular bone structure, provides an ideal environment for the survival of bone metastatic cells (27). The slow blood flow in sinusoid vessels further facilitates the colonization of the bone marrow by hematopoietic stem cells and invasive tumor cells. Additionally, endothelial cells in sinusoidal vessels express a variety of adhesion molecules, including E-selectin, P-selectin, intracellular adhesion molecules and vascular cell adhesion molecules (VCAMs), which promote the homing of tumor cells to the bone marrow (28-30). Following tumor colonization of the bone, the bone microenvironment facilitates tumor growth and invasion. Various resident and transient cells, including stromal cells, osteoblasts and immune cells, affect tumor survival. Stromal cells, which originate from mesenchymal cells within the bone marrow and include adipocytes, fibroblasts and osteoblasts, promote tumor cell proliferation and differentiation via the secretion of VCAMs, syndecan and matrix metalloproteinase 2 (MMP-2) (31). Osteoclast-mediated osteolysis further promotes tumor growth by releasing growth factors from the bone, which increase tumor cell proliferation and osteolysis. Transient cells, including red blood cells, platelets, and T cells, also promote tumor growth and metastasis via a variety of pathways and molecular interactions (28).

RANK-RANKL-OPG system. The RANK-RANKL-OPG system plays a crucial role in the promotion of cancer cell proliferation, epithelial-mesenchymal transition (EMT) and bone metastasis (32,33). Tumor cells secrete various cytokines within the bone microenvironment that affect the RANK-RANKL-OPG system (34). Tumor-derived parathyroid hormone-related protein (PTHrP), insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF) and platelet-derived growth factor not only increase tumor cell growth in an autocrine manner but also promote the production and release of RANKL by osteoblasts and stromal cells (35). In addition, tumor-derived PTHrP, IL-1, prostaglandin E2, BMP and epithelial growth factor downregulate OPG expression in

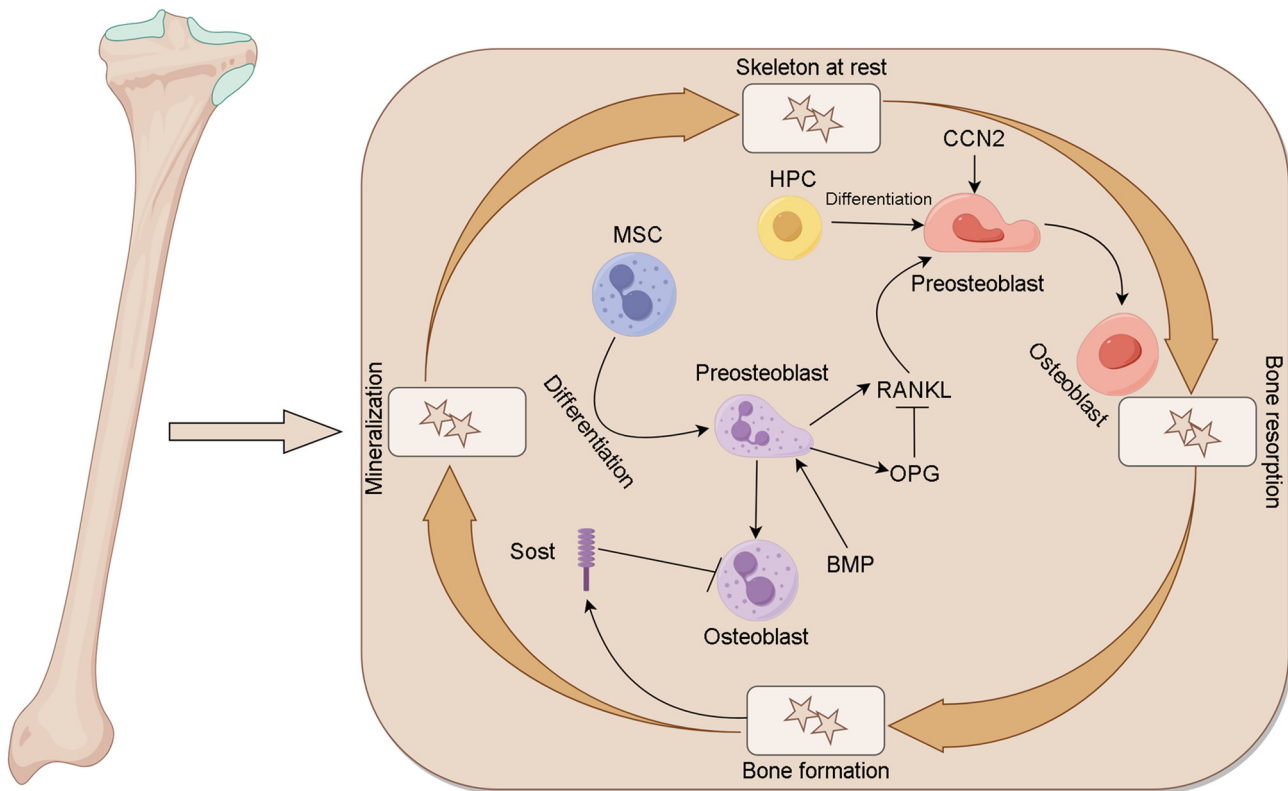


Figure 1. Dynamic cycle of bone remodeling. Stars indicate the state of the bone in the bone microenvironment. Created with BioRender.com. MSC, mesenchymal stem cell; BMP, bone morphogenetic protein; Sost, sclerostin; HPC, hematopoietic stem cell; RANKL, receptor activator of nuclear factor- κ B ligand; OPG, osteoprotegerin; CCN2, cellular communication network factor 2/connective tissue growth factor.

the stroma and osteoblasts (36). Accordingly, RANKL levels increase and OPG levels decrease in the tumor bone microenvironment, which disrupts the dynamic balance of bone remodeling. The binding of RANKL to RANK promotes the fusion, differentiation and maturation of osteoclast precursors through mitogen-activated protein kinase (MAPK) and nuclear factor- κ B signaling pathways, and enhances osteolysis through c-Src signaling (37,38). In addition, PTHrP stimulates the secretion of MMP-13 via the protein kinase C (PKC)-ERK signaling pathway, and MMP-13 contributes to bone degradation and bone fractures (39).

Transforming growth factor- β (TGF- β) plays a dual role. TGF- β acts as a suppressor in the early stages of tumorigenesis but promotes tumor progression in later stages (40). Bone metastasis occurs at an advanced stage of cancer, and TGF- β plays a key role in bone metastasis and the promotion of tumor development. TGF- β is primarily released into the bone microenvironment by osteolysis, and mediates the EMT, invasion, angiogenesis and immunosuppression of tumor cells via TGF- β /Smad signaling (41,42). Integrin α v β 3 has been demonstrated to be required for the TGF- β /Smad signaling that triggers breast cancer metastasis (43), but the underlying mechanism remains to be further elucidated. Tumor cells undergoing EMT experience cytoskeletal rearrangement and loss of intercellular adhesion, ultimately increasing their invasion, motility and metastatic potential. Preclinical experiments have shown that TGF- β inhibitors have the ability to inhibit bone metastases in animal models of breast cancer, but their effectiveness is limited in lung cancer (44). These findings

suggest that the mechanism of TGF- β in bone metastases varies according to the primary tumor type, highlighting that further studies are necessary to classify primary tumors based on their mechanism of metastasis.

Mechanisms of osteoblastic metastasis

Wnt-LRP5- β -catenin pathway. Tumor cells interact with osteoblasts, leading to the production of TGF- β , BMP, IGF and FGF. This interaction also promotes Wnt signaling, which induces osteogenic activity (Fig. 3). TGF- β and BMP activate Smad signaling in osteoblasts, while growth factors activate MAPK and PKC signaling in osteoclasts. In addition, Wnt activates the β -catenin regulatory signaling pathway. These pathways converge and interact with the RUNX2 transcriptional network to induce osteoblast differentiation and proliferation. Research has shown that the Wnt/ β -catenin pathway is crucial for osteoblast function and bone formation, with activation of this pathway stimulating osteoblast development and promoting bone formation (45). Osteocytes secrete Sost, which binds to LRP5, thereby blocking the Wnt-LRP5- β -catenin pathway and inhibiting bone formation. The serum level of Sost is upregulated in patients with multiple myeloma, as myeloma cells secrete Sost; therefore, patients with multiple myeloma frequently present with osteolytic lesions. *In vitro* data suggest that breast cancer cells induce Sost expression to inhibit bone metastasis and osteogenesis; however, in prostate cancer, Sost expression is downregulated while BMP-6 expression is upregulated (46).

Dickkopf-1 (DKK-1) can also bind to LRP5 to inhibit Wnt signaling (47). Endothelin-1 (ET-1) and PTHrP mediate bone

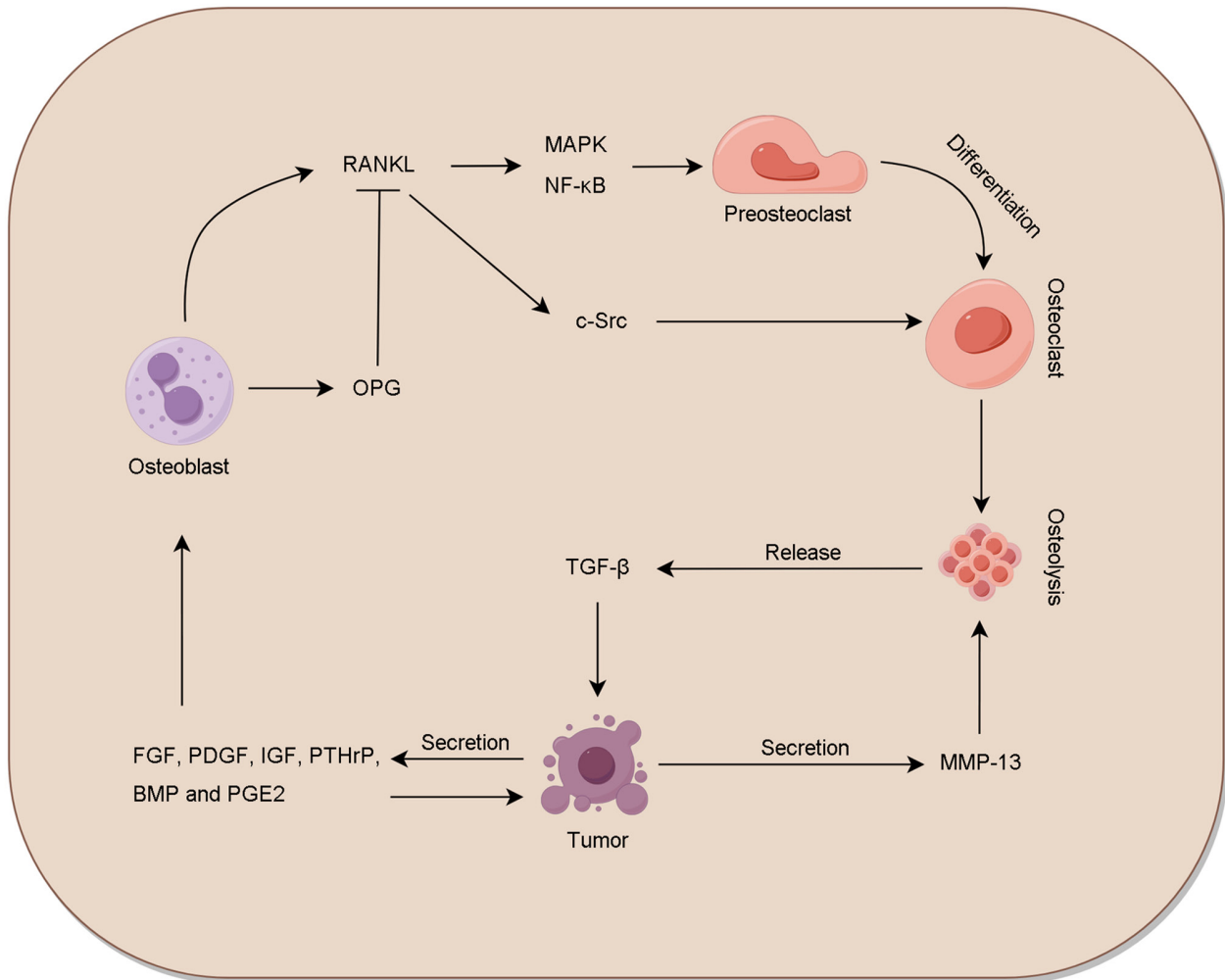


Figure 2. Mechanisms of osteolytic metastasis. Created with BioRender.com. RANKL, receptor activator of nuclear factor- κ B ligand; MAPK, mitogen-activated protein kinase; TGF- β , transforming growth factor- β ; MMP-13, matrix metalloproteinase 13; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; PTHrP, parathyroid hormone-related protein; BMP, bone morphogenetic protein; PGE2, prostaglandin E2; OPG, osteoprotegerin.

formation. ET-1 is one of four isoforms of ET, the activity of which is regulated by the proteolytic conversion of an inactive precursor to an active form (48). Prostate epithelial cells and most prostate cancer cell lines have been indicated to secrete a substantial amount of ET-1, and plasma ET-1 concentrations are elevated in patients with advanced prostate cancer. ET-1 secreted by prostate bone metastases binds to ET receptor A on osteoblasts, which stimulates their proliferation and osteogenesis via β -catenin and MAPK (49). Activated osteoblasts in the bone microenvironment release factors that stimulate tumor cell proliferation and invasion (50). In addition, tumor cell-derived ET-1 promotes osteogenesis in osteoblasts via the inhibition of DKK-1 synthesis (51). Notably, the N-terminal fragment of PTHrP can mimic the binding of ET-1 to the endothelin receptors in osteoblasts to promote bone formation (52). Furthermore, prostate specific antigen (PSA) can suppress the osteolytic effect of PTHrP (53).

RANK-RANKL-OPG system. Downregulation of the RANKL-to-OPG ratio in the RANK-RANKL-OPG system can inhibit osteolysis and lead to an osteogenic phenotype. In prostate cancer, PSA has been shown to inhibit osteolytic activity by inhibiting the expression of RANKL in osteoblasts

and promoting the function of osteoblasts (54). In addition, the expression levels of RANK and RANKL in tumor tissue from patients with metastatic prostate cancer are significantly higher compared with those in patients with local disease (55). Notably, RANKL-induced osteolysis appears to facilitate the colonization of osteoblastic bone metastatic tumor cells (56). The ability of RANKL to promote the intraosseous growth of prostate cancer cells has been found to be associated with IGF signaling and hypoxia-inducible factors, which create a bone microenvironment favorable for tumor growth (57).

In general, the mechanisms underlying the behavior of cancer cells in bone metastasis are highly complex and largely unknown, despite decades of research. Additional research, as well as effective and suitable animal models, are required to elucidate the specific mechanisms and identify novel therapeutic targets.

4. Animal models of cancer bone metastasis

Cancer is the second leading cause of human death worldwide, after cardiovascular diseases, with metastases typically being the cause of mortality and bone being the most prevalent site of

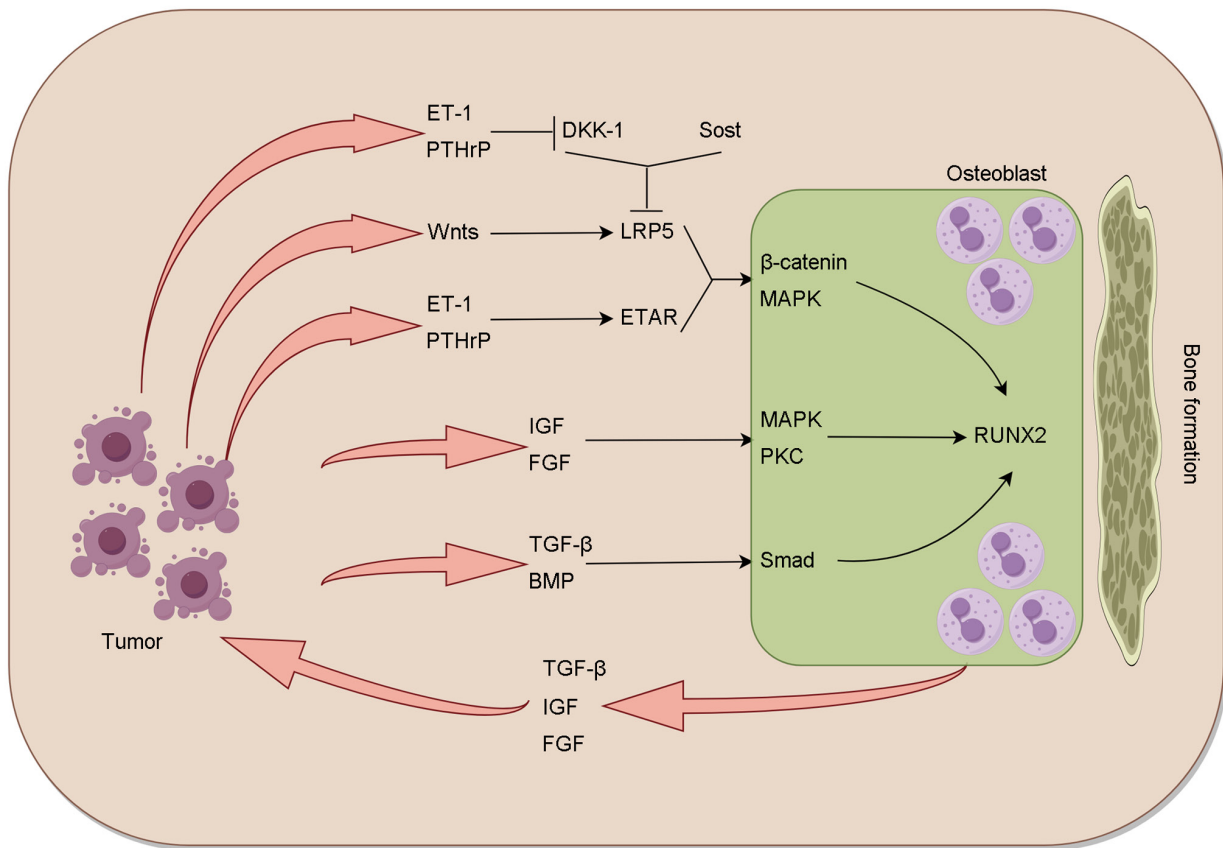


Figure 3. Mechanisms of osteogenic metastasis. Created with BioRender.com. ET-1, endothelin-1; PTHrP, parathyroid hormone-related protein; DKK-1, Dickkopf-1; Sost, sclerostin; LRP5, lipoprotein receptor-associated protein 5; ETAR, endothelin A receptor; IGF, insulin-like growth factor; FGF, fibroblast growth factor; TGF- β , transforming growth factor- β ; BMP, bone morphogenetic protein; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; RUNX2, Runt-related transcription factor 2.

metastasis (58). Bone metastases are common among patients with breast, prostate or lung cancer, and may also occur in patients with other tumors, including myeloma, renal and thyroid cancers, Ewing's sarcoma and lymphoma (59,60). The general pathogenesis of bone metastasis involves several stages, including primary tumor proliferation, local tissue invasion, intravascular invasion, extravasation into the bone marrow, tumor cell dormancy, intraosseous proliferation and changes in the intraosseous microenvironment. In 1889, Stephen Paget proposed the 'seed and soil' hypothesis (61). This hypothesis proposes that the interaction between cancer cells and the organ microenvironment influences cancer cell proliferation, survival and expansion, and that the ability of the cancer cells to recruit a blood supply determines whether the cancer cells will metastasize. Understanding the molecular pathways involved in cancer metastasis is crucial for preventing the formation and growth of bone metastases. Animal models of bone metastasis are essential for investigating these molecular pathways. An ideal animal model should be clinically relevant, mimic human disease, and reproducible. However, each model has both advantages and disadvantages, and no single model is perfect. Researchers must choose the most appropriate model based on the specific research question.

Advantages and disadvantages of different routes of inoculation for modeling cancer bone metastasis. The injection techniques that are commonly used to study bone

metastasis include tail vein, intracardiac, intraosseous, orthotopic, subcutaneous and tail artery injections. Each type of bone metastasis model offers unique benefits and limitations. Therefore, it is critical to choose a model that is suitable for the specific research direction and purpose.

Tail vein injection is a relatively simple method of injection that is primarily used in studies of tumor blood circulation and lung metastasis. This injection method rarely leads to bone metastasis but more frequently leads to lung metastasis (62).

Intracardiac inoculation typically involves injecting tumor cells into the left ventricle of mice, allowing the cells to circulate systemically and spread to the bone to establish bone metastases. This type of model has a high tumorigenicity and a short modeling time, allowing the distribution of tumor cells to be monitored in real-time using bioluminescence imaging when fluorescently labeled tumor cells are injected. It is the most frequently used animal model of bone metastasis. However, the successful construction of this model is challenging. In addition, as tumor cells spread throughout the body via the systemic circulation, multiple metastases can develop, sometimes leading to fatal non-bone metastases before bone metastasis occurs, which may complicate the specific study of bone metastasis (63).

Intraosseous injection involves directly injecting tumor cells into the femur or tibia, leading to tumor formation in the bone. Compared with left ventricular injection, intraosseous injection results in a higher incidence of bone metastasis and

a shorter experimental period. However, it also disrupts the integrity of the bone, which makes it less suitable for studying the molecular mechanism of bone metastasis (64). In addition, as it does not involve metastasis, with the movement of cells from a primary site to the bone, this model is not strictly a model of metastasis. However, it can be used for the investigation of tumor-bone interactions.

Orthotopic inoculation is a surgical method in which tumor cells or tumor masses are directly inoculated into the organs of mice, such as the prostate and mammary glands. Compared with the spontaneous canine bone metastasis model, this method has greater tumorigenicity and shorter latency while retaining the biological characteristics of tumor cells. It can effectively simulate the whole process of tumor metastasis from the primary site to the bone. However, in numerous cases, mice in this model develop lung and lymph node metastases before bone metastasis, and succumb to other causes before bone metastases can develop.

Subcutaneous inoculation is a method of injecting tumor cells or tumor fragments into the skin of mice to form tumors. This method is simple and easy to manage; however, it rarely causes bone metastasis, even when using cells derived from the bone metastases of patients (65). Currently, subcutaneous tumor models are primarily derived from patient samples and are used to evaluate the inhibitory effects of antitumor drugs against human tumors (66).

Caudal artery injection (63) delivers tumor cells directly into the tail artery of a mouse. This method is relatively easy to perform and effectively transfers cancer cells to the posterior limb bone. It is more efficient than intracardiac injection, and markedly shortens the time taken for bone metastasis to develop. Furthermore, tail artery injection rarely causes acute mortality, which allows researchers to inject a higher number of cancer cells, which accelerates the development of bone metastasis for multiple cell lines. However, this method may cause some tumor cells to metastasize to the lung, which complicates the study of bone metastasis. Table I summarizes the methods used to model bone metastasis via the caudal artery injection of tumor cells (63,67-71).

Cell lines and methods for establishing animal models of cancer bone metastasis
Prostate cancer. To date, dogs are the only nonrodent animals known to develop spontaneous prostate cancer. However, their use in research is challenging due to difficulties in experimental control, the low incidence of spontaneous tumor formation and bone metastasis, and the high cost (72). Lobund-Wistar rats, ACI/segHapBR rats, C57BL/6 mice, BALB/c nude mice, severe combined immunodeficiency mice and various transgenic mouse models have been used to investigate bone metastasis in prostate cancer (73-75). However, bone metastasis in rat prostate cancer models is very rare. Due to their short experimental period, low economic cost and research convenience, mice have become the most commonly used animals for studying prostate cancer bone metastasis.

One of the most commonly used prostate cancer cell lines is PC3, which was isolated in 1979 from a bone metastatic lesion in a 62-year-old White male patient with grade IV prostate cancer (76). This cell line has high metastatic potential and exhibits characteristics more typical

of neuroendocrine carcinoma than adenocarcinoma (77). Numerous PC3 cell sublines now exist, and PC3 cells can be reimplanted *in vivo* to screen for highly metastatic variants. In 1984, Kozlowski *et al* (78) injected PC3 cells into nude mice and subsequently harvested metastatic cells from the liver, which were designated PC3M cells. Later, in 1996, Pettaway *et al* (79) orthotopically injected PC3M cells into the prostate of mice, and subsequently isolated metastatic cells from the lymph nodes and used them to establish a PC3M-LN4 cell subline.

DU145 cells were originally derived from the brain of a 69-year-old White male patient with metastatic prostate cancer (80). In immunodeficient mice, the intratibial or intracardiac injection of DU145 cells leads to the formation of lytic bone lesions (77). DU145 cells offer advantages in the study of prostate cancer, as their resemblance to adenocarcinoma is closer than that of PC3 cells, and they are capable of producing bone metastases *in vivo*. DU145 cell-derived bone metastases, like those of PC3, are osteolytic (77).

LNCaP cells were established by Horoszewicz (81) in 1977 via isolation from the left supraclavicular lymph node of a 50-year-old White male patient with metastatic prostate cancer. In a subsequent study, LNCaP cells were inoculated in castrated mice until bone metastases developed; these resulting cells were collected and designated as LNCaP C4-2B (82). LNCaP C4-2B cells exhibit androgen-independent characteristics and have greater metastatic potential than the parental LNCaP cell line, as they form mixed osteolytic-osteogenic lesions in immunodeficient mice when injected via intraosseous or intracardiac routes (77,83).

Breast cancer. Bone is one of the most prevalent sites for breast cancer metastasis in females. Bone metastases in breast cancer are often osteolytic and associated with high morbidity and mortality rates; ~80% of patients with advanced breast cancer develop bone metastases (84). The median survival time of patients with breast cancer bone metastasis is only 36 months (85). However, animal models have contributed to improvements in the treatment of bone metastasis in patients with breast cancer (86). Mice, rats, cats and dogs frequently develop spontaneous benign and malignant mammary tumors. However, these spontaneous breast cancers are usually unsuitable for studying bone metastasis as most spontaneous breast tumors in mice and rats do not metastasize, instead causing only local invasion (87). Furthermore, the majority of breast adenocarcinomas in rodents rapidly lose estrogen responsiveness (88), making them unsuitable models for the study of estrogen-responsive female breast cancer.

MDA-MB-231 cells were originally isolated from the breast of a 40-year-old White female patient with breast cancer, and are commonly used in bone metastasis research. MDA-MB-231 and its bone metastatic subline have been used in intracardiac, *in situ*, intraosseous and caudal vein injection studies. These cells metastasize almost entirely to the bone and cause osteolytic metastasis 3-4 weeks after injection. Bisphosphonates have been demonstrated to reduce the formation of new bone metastases from MDA-MB-231 in animal models, and to hinder the growth of existing bone metastases (89).

MCF7 cells are breast adenocarcinoma cells isolated from the pleural effusion of a 69-year-old White female patient.

Table I. Mouse models of cancer bone metastasis using caudal artery injection.

First author, year	Type of cancer	Cell line	No. of cells	Mouse strain	(Refs.)
Kuchimaru <i>et al</i> , 2018	Breast cancer	4T1/luc	1x10 ³	Female BALB/c	(63)
Farhoodi <i>et al</i> , 2020	Breast cancer	4T1/luc	5x10 ³ -5x10 ⁴	Female BALB/cJ	(67)
Kuchimaru <i>et al</i> , 2018	Breast cancer	MDA-MB-231/luc	5x10 ⁵	Female NOD-SCID	(63)
Winnard <i>et al</i> , 2024	Breast cancer	MDA-MB-231/luc	5x10 ⁵	Female NOD-SCID	(68)
Kuchimaru <i>et al</i> , 2018	Breast cancer	E0771/mKO-luc	2x10 ⁵	Female C57B/6 albino	(63)
Kuchimaru <i>et al</i> , 2018	Breast cancer	MCF7	1.5x10 ⁶	Female SCID	(63)
Han <i>et al</i> , 2018	Breast cancer	HCC-2218	5x10 ⁵	Female NOD	(69)
Kuchimaru <i>et al</i> , 2018	Lung cancer	LLC/luc	2x10 ⁵ or 1x10 ⁶	Male C57B/6	(63)
Kuchimaru <i>et al</i> , 2018	Prostate cancer	PC3/luc	1x10 ⁶	Male SCID	(63)
Ye <i>et al</i> , 2023	Prostate cancer	RM1	1x10 ⁵	Male C57/BL6J	(70)
Zhong <i>et al</i> , 2020	Prostate cancer	MPC3/luc	2.9x10 ⁵	Male C57BL/6J	(71)
Kuchimaru <i>et al</i> , 2018	Renal carcinoma	786-O/luc	1x10 ⁶	Male BALB/c-nu	(63)

NOD, non-obese diabetic; SCID, severe combined immunodeficiency; nu, nude.

These cells express estrogen receptors and can generate mixed osteolytic or osteogenic bone metastases following intraosseous injection. Bone metastasis develops gradually, potentially taking up to 6 months after intracardial injection (90).

ZR-75-1 cells were isolated from the breast tissue of a 63-year-old White female patient with ductal breast cancer. These cells have been used to establish an osteoblastic breast cancer bone metastasis model in nude mice via intracardiac injection. This model was used to demonstrate the critical involvement of ET-1 in the pathophysiology of osteoblastic metastasis (91).

4T1 is a mammary tumor cell line derived from spontaneous mammary tumors in BALB/c mice. These cells are highly invasive and tumorigenic, with growth and metastatic spread patterns very similar to those of human breast cancer (92). 4T1 cells can easily be transplanted into the mammary gland, allowing the primary tumor to grow in the anatomically correct site, closely mimicking the metastatic pattern observed in human breast cancer. In addition, the course of 4T1 metastasis to lymph nodes and other organs is very similar to that of human breast cancer (93). Tumors often form on days 7-10 following the orthotopic injection of 4T1 cells into the mammary fat pads of female BALB/c mice, with metastasis to the bone and internal organs occurring 3-4 weeks later. Although the incidence of bone metastasis following the orthotopic injection of 4T1 cells can reach 100%, the reliability of this model is poor (94).

Lung cancer. Lung cancer is classified into two subtypes: Small cell lung cancer and non-small cell lung cancer, each with distinct biological behaviors, clinical courses and treatment responses. Bone metastases occur in 30-40% of patients with lung cancer (95). These metastases are primarily osteolytic lesions, osteoblastic lesions and mixed bone lesions (96,97). Several cell lines, including A549, ACC-LC319, H460, H727, H2030, HARA, LLC, SBC-3, NCI-H292, PC9, PC14, SBC-5 and SPC-A-1, have been used in lung cancer bone metastasis animal models (98).

Orthotopic, tail vein, intraosseous and intracardiac injections have all been used in models of bone metastasis in lung cancer; however, orthotopic injection more closely replicates the biological behavior of lung cancer metastasis compared with other methods. Intraosseous injections can be administered to the tibia or spinal canal, and the direct injection of PC14 cells into the spinal canal of mice was found to result in spinal metastases of lung cancer (99). The osteoporosis and spinal compression caused by bone metastases in this model closely resemble those observed with human spinal metastases. Certain cell lines can be selectively cultured *in vivo* to generate sublines that more readily metastasize to the bone. For example, although the bone metastasis of PC14 cells in mice is rare, multiple *in vivo* selective cultures enabled a highly metastatic PC14HM subline to be established (100). Similarly, following eight cycles of *in vivo* selection of the SPC-A-1 cell line, the SPC-A-1-BM cell subline achieved a bone metastasis success rate of 100% in mice following intracardiac injection (101). Table II summarizes the specific conditions of the cell lines and modeling methods commonly used for studying bone metastases (77,85,86,102-120).

5. Conclusion and perspectives

Direct cell injection is the most frequently used approach for the development of bone metastasis models, with intracardiac and intraosseous injection methods being the most successful in bone metastasis research. Tail vein injection is simpler than cardiac injection but frequently leads to lung metastasis, which complicates the study of bone metastasis. Although cell injection models are straightforward and easy to maintain, they provide limited information about the process by which tumors metastasize to bone. Animal models are very important in the study of the pathogenesis of bone metastasis. The development of different animal models for different research purposes and research directions is critical. The identification of key targets for the treatment of bone metastasis may improve therapy

Table II. Cell lines and modeling methods for common cancer bone metastasis.

First author, year	Type of cancer	Cell line	Type of bone metastasis	Inoculation route	Mouse strain	No. of cells	(Refs.)
Gravina <i>et al.</i> , 2015	Prostate cancer	PC3	Osteolytic	Intracardiac	Nude	1x10 ⁵	(102)
Wu <i>et al.</i> , 1998	Prostate cancer	PC3	Osteolytic	Intracardiac	SCID/bg mice	5x10 ⁵	(103)
Wu <i>et al.</i> , 1998	Prostate cancer	PC3	Osteolytic	Intraosseous	SCID	5x10 ⁵	(103)
Shevrin <i>et al.</i> , 1988	Prostate cancer	PC3	Osteolytic	Intravenous	Nude	1x10 ⁶	(104)
Havens <i>et al.</i> , 2008	Prostate cancer	PC3	Osteolytic	Orthotopic or subcutaneous	SCID	2x10 ⁵	(105)
Yang <i>et al.</i> , 1999	Prostate cancer	PC3	Osteolytic	Orthotopic	Nude	1 mm ³ of a subcutaneous tumour	(106)
Fisher <i>et al.</i> , 2002	Prostate cancer	DU145	Osteolytic	Intraosseous	Nude	7.7x10 ⁴	(107)
Bonfil <i>et al.</i> , 2007	Prostate cancer	DU145	Osteolytic	Intraosseous	SCID	1x10 ⁵	(108)
Zou <i>et al.</i> , 2013	Prostate cancer	LNCaP	Osteoblastic	Intracardiac	Nude	5x10 ⁵	(109)
Bonfil <i>et al.</i> , 2007	Prostate cancer	LNCaP	Osteoblastic	Intraosseous	SCID	2x10 ⁵	(108)
Corey <i>et al.</i> , 2002	Prostate cancer	LNCaP	Osteoblastic	Intraosseous	SCID	1x10 ⁵	(110)
Jantscheff <i>et al.</i> , 2009	Prostate cancer	LNCaP	Osteoblastic	Orthotopic	SCID	1x10 ⁶	(111)
Wu <i>et al.</i> , 1998	Prostate cancer	C4-2	Osteoblastic	Intracardiac or intraosseous	Nude and SCID/bg	5x10 ⁵ intracardiac or 1x10 ⁶ intraosseous	(103)
Thalmann <i>et al.</i> , 1994	Prostate cancer	C4-2	Osteoblastic	Orthotopic or subcutaneous	Castrated and intact nude	1x10 ⁶	(82)
Wetterwald <i>et al.</i> , 2002	Breast cancer	MDA-MB-231	Osteolytic	Intraosseous	BALB/c-nu/nu	1x10 ⁴	(112)
Sasaki <i>et al.</i> , 2021	Breast cancer	MDA-MB-231	Osteolytic	Intraosseous	SCID	1x10 ⁶	(113)
Wetterwald <i>et al.</i> , 2002	Breast cancer	MDA-MB-231	Osteolytic	Intracardiac	BALB/c-nu	1x10 ⁵	(112)
Kim <i>et al.</i> , 2020	Breast cancer	MDA-MB-231	Osteolytic	Intracardiac	BALB/c-nu	1x10 ⁵	(114)
Yoneda <i>et al.</i> , 2000	Breast cancer	MDA-MB-231	Osteolytic	Intracardiac	BALB/c-nu	5x10 ⁵	(115)
Yin <i>et al.</i> , 2003	Breast cancer	ZR-75-1	Osteoblastic	Intracardiac	Nude	1x10 ⁵	(91)
Ooi <i>et al.</i> , 2010	Breast cancer	MCF7	Osteoblastic	Intraosseous	Nude	5x10 ⁴	(90)
Yi <i>et al.</i> , 2002	Breast cancer	MCF7	Osteoblastic	Intracardiac	Nude	5x10 ⁵	(116)
Sun <i>et al.</i> , 2019	Breast cancer	4T1	Osteolytic	Orthotopic	BALB/c	1x10 ⁵	(117)
Sasaki <i>et al.</i> , 2021	Breast cancer	4T1	Osteolytic	Intraosseous	BALB/c	5x10 ³	(113)
Sun <i>et al.</i> , 2019	Breast cancer	4T1	Osteolytic	intracardiac	BALB/c	1x10 ⁴	(117)
Hung <i>et al.</i> , 2014	Lung cancer	A549	Osteolytic	Intracardiac	NOD/SCID	5x10 ⁵	(118)
Liu <i>et al.</i> , 2020	Lung cancer	A549	Osteolytic	Intravenous	SCID	1.8x10 ⁶	(119)
Wang <i>et al.</i> , 2023	Lung cancer	H460	Mixed	Intraosseous	BALB/c	1.0x10 ⁶	(120)

NOD, non-obese diabetic; SCID, severe combined immunodeficiency; bg, beige; nu, nude.

efficacy and patient quality of life, and ultimately extend patient survival.

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Availability of data and materials

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Authors' contributions

MD, JG and GQ were responsible for conception and design. JG and GQ supervised the study. MD, YZ and HD wrote the original manuscript. JG, GQ and MD reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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