

Pathophysiology of Bone Remodelling and Current Therapeutic Approaches

Molekulare Grundlagen des Knochenmilieus und klinische Konsequenzen

Authors

I. Juhasz-Böss¹, T. Fehm², J. T. Ney¹, E. F. Solomayer¹

Affiliations

¹ Klinik für Frauenheilkunde, Geburtshilfe und Reproduktionsmedizin, Universitätsklinikum des Saarlandes, Homburg/Saar
² Universitätsfrauenklinik Tübingen, Tübingen

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Correspondence

Dr. Ingolf Juhasz-Böss
 Universitätsklinikum des Saarlandes
 Klinik für Frauenheilkunde, Geburtshilfe und Reproduktionsmedizin
 Homburg/Saar
 Ingolf.Juhasz-Boess@uniklinikum-saarland.de

Abstract

Different metabolic bone parameters such as RANK (receptor activator of nuclear factor- κ B), RANK ligand (receptor activator of nuclear factor- κ B ligand) and OPG (osteoprotegerin) control physiological bone remodelling. The pathophysiology of these factors in bone diseases and osseous metastases is becoming clearer. In metastatic breast cancer, osteolytic bone metastases are the result of increased osteoclastic activity caused either by increased RANK ligand or decreased OPG expression of metastatic osseous tumour cells. These findings may lead to new therapeutic options for the treatment of breast cancer patients. The aim of this work is to provide an overview of physiological bone remodelling and of the interaction between tumour cells and bone environment. Current therapy approaches and the mechanisms of action of drugs are described.

Zusammenfassung

Das physiologische Knochen-Remodelling unterliegt u. a. der Steuerung durch die Knochenstoffwechsellparameter RANK (receptor activator of nuclear factor- κ B), RANK-Ligand (receptor activator of nuclear factor- κ B ligand) und OPG (osteoprotegerin). Auch die pathophysiologische Beteiligung dieser Faktoren bei ossär bedingten Erkrankungen wird zunehmend klarer. Beim Mammakarzinom wird die Entstehung von osteolytischen Knochenmetastasen durch eine gesteigerte Osteoklastenaktivität verursacht, die durch übermäßige direkte RANK-Ligand- oder reduzierte OPG-Expression ossär metastasierter Tumorzellen mitbedingt ist. Diese Erkenntnisse ermöglichen nebst dem Einsatz etablierter Therapieverfahren neue Therapieoptionen in der Behandlung von Mammakarzinompatientinnen. Ziel dieser Arbeit ist es, einerseits einen Überblick über das physiologische Knochen-Remodelling zu geben. Zudem soll die Wechselwirkung zwischen Tumorzelle und dem Knochenmilieu dargestellt werden. Ferner sollen aktuelle Therapieansätze vorgestellt und Wirkmechanismen der Therapeutika näher erläutert werden.

Physiological Bone Remodelling

Bones consist of 3 types of bone cells. Osteoclasts are responsible for bone resorption and originate from haematopoietic stem cells. Osteoblasts are responsible for bone formation and originate from mesenchymal stem cells. Osteocytes are the 3rd type of bone cell and the most numerous cells (ca. 90%) found in bone; they bear a resemblance to the inactive osteoblasts which form part of the bone matrix.

Bone remodelling is regulated by numerous hormones, cytokines and growth factors. These factors are capable of either decreasing or increasing

bone mass. Factors responsible for decreasing bone mass include parathyroid hormone (PTH), corticosteroids and tumour necrosis factors (TNF), while factors which contribute to increasing bone mass primarily include calcitonin, estrogens and androgens as well as transforming growth factor beta (TGF-beta) [1,2].

Bone metabolism is a complex process which largely depends on the interplay between RANK ligand (receptor activator of nuclear factor- κ B ligand), RANK (receptor activator of nuclear factor- κ B) and osteoprotegerin (OPG). RANK ligand, which is expressed by osteoblasts, is one of the most important mediators of bone resorption.

RANK ligand is also expressed by T-lymphocytes, dendritic cells and even tumour cells. RANK ligand binds to RANK, a member of the family of tumour necrosis factor receptors, and is located on osteoclast precursor cells where it promotes the development and activation of osteoclasts (● Fig. 1).

Active osteoclasts break down bone tissue. The resultant decrease in bone is compensated by bone formation generated by osteoblasts [2]. Numerous factors stimulate osteoblast expression of RANK ligand: prostaglandins (PGE), PTH, glucocorticoids, vitamin D, interleukins (IL-1, IL-6, IL-11), parathyroid hormone-related peptide (PTHrP) and TNF-alpha [2, 11].

OPG inhibits the binding of RANK ligand to RANK. This prevents the fusion of osteoclast precursors to form multi-nucleated active osteoclasts, thus inhibiting bone resorption. Osteoclast activity is determined by the ratio of RANK ligand to OPG. High levels of OPG prevent osteoclast activation, while high amounts of RANK ligand promote their activation. Changes to the RANK ligand/OPG ratio are a crucial factor in the pathogenesis of bone diseases arising from increased bone resorption [2, 11, 13].

Tumour/Bone Interactions

Tumour cells express a number of factors which activate osteoblasts to express RANK ligand, including PTHrP, cytokines (IL-11) and growth factors (TGF- β). These factors stimulate osteoblasts and other bone cells to increase their expression of RANK ligand. The over-expression of RANK ligand leads to increased formation and function of osteoclasts as well as longer osteoclast survival times, resulting in excessive bone resorption. During bone resorption, the bone matrix releases the growth factors TGF- β , VEGF (vascular endothelial growth factor) and IGF (insulin-like growth factor), which stimulate tumour activity. This can lead to a vicious circle of bone destruction and tumour growth [14, 18]. The process of osteolysis depends on numerous factors. Some factors originate from serum, others from the tumour cell itself, and yet others from osteoblasts.

Most factors target the osteoblast, with the main effect occurring through the increased release of RANK ligand [3]. Factors which target the osteoblast include PTH, PTHrP, cyclooxygenase 2 (COX-2), IL-1, IL-11, TNF-alpha, insulin-like growth factors (IGF), TGF-beta, etc.

However, a few factors do not target osteoblasts but osteoclasts. They can directly affect osteoclastogenesis by either inhibiting or increasing it. Factors which increase osteoclastogenesis include interleukins (IL-6, IL-8), macrophage-colony stimulating factors (M-CSF) and vascular endothelial growth factors (VEGF). In addition to osteoblasts and osteoclasts, the matrix can also be directly involved in osteolysis [3].

The more than 20 known matrix metalloproteinases (MMPs) are important factors in physiological bone remodelling. However, the main enzyme in bone lysis is cathepsin K, which is released by osteoclasts and leads to a degeneration of the bone matrix in the contact area between osteoclast and bone matrix. Bone matrix consists of $\frac{2}{3}$ hydroxylapatite crystals and $\frac{1}{3}$ collagen, primarily type-1 collagen. The environment at the contact area between the osteoclast and the bone surface is acidic, which decreases the activity of MMPs in this area. Cathepsin K, which is released by osteoclasts, is the main lytic factor and primarily responsible for the lysis of type-1 collagen [3].

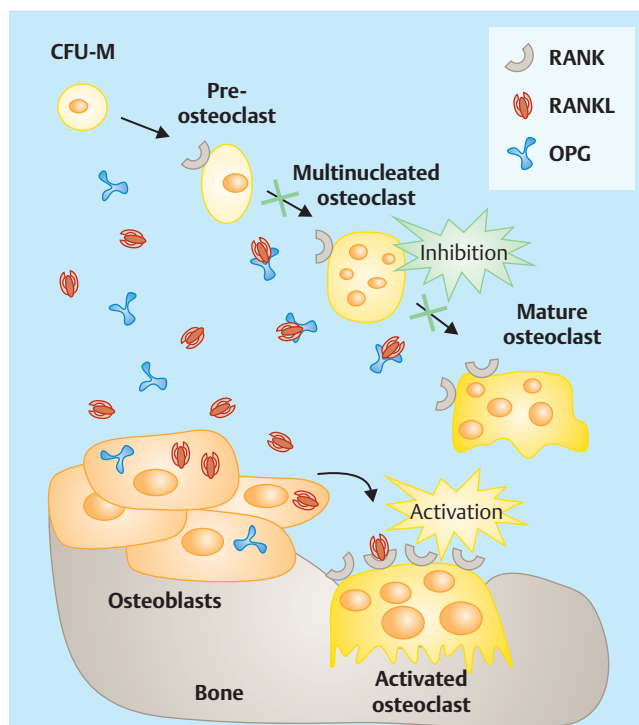


Fig. 1 Physiological bone remodelling showing osteoblast-osteoclast interaction through the RANK, RANK ligand, and OPG systems (according to Ney et al. [15]).

RANK, RANK Ligand and OPG in Breast Cancer

Numerous studies have been published which have investigated the expression of RANK, RANK ligand and OPG in breast cancers. Indications were found that these factors are expressed in breast cancer cell lines. With regard to the primary tumour, however, the data on the expression of RANK, RANK ligand and OPG is unclear. It should be noted that there are only a few studies with relatively low numbers of cases which have investigated this point. The methodology used to investigate the 3 factors has also been inconsistent [15].

In 2011, Santini et al. investigated the expression of RANK, RANK ligand and OPG at the mRNA level and immunohistochemically at the protein level for RANK in one of the largest collectives studied to date [20]. Microarray investigations of almost 300 breast cancers detected the presence of RANK as well as of RANK ligand and OPG in the primary tumour. The expression of these factors depending on the type of breast cancer was also studied. Basal breast cancers were found to express RANK mRNA at significantly higher levels compared to non-basal breast cancers.

The breast cancer collective was also divided into risk groups according to tumour size (<2 cm or >2 cm) and according to grading and hormone status. It was notable that women with tumour sizes >2 cm had significantly higher levels of RANK mRNA than patients with tumour sizes <2 cm. When the correlation with grading was investigated, it was found that patients with G3 tumours had significantly higher RANK mRNA expression in the primary tumour than patients with G1 and G2 tumours, and patients with G3 tumours had a significantly lower OPG expression compared to patients with G1 and G2 tumours.

The authors also divided the collective into breast cancers according to prognosis. G3 tumours and tumours which were > 2 cm and estrogen-receptor negative were defined as having a worse prognosis. It was notable in this group patients had high levels of RANK mRNA expression and low levels of OPG mRNA expression.

The extent of bone metastasis correlated to the level of RANK expression was also investigated. Here again, it was notable that bone metastasis was significantly increased and occurred earlier in patients with high levels of RANK expression in the primary tumour.

The authors also investigated whether RANK, RANK ligand and OPG could also be independent prognostic factors. For this the group was divided into patients with low, medium and high levels of RANK and OPG expression. Patients with higher levels of RANK expression had poorer disease-free survival (DFS) rates and a significantly poorer overall survival (OS) rate compared to patients with lower levels of RANK expression ($p=0.059$ and $p=0.0078$, respectively). Analogously, patients with higher OPG expression had significantly better rates for disease-free survival and overall survival compared to patients with low OPG expression ($p=0.0402$ and $p=0.0335$, respectively) [20].

That increased OPG expression appears to be correlated with a better prognosis was also shown by Ruckhaeberle et al. in a poster presented at the SABCS 2011. Gene expression analysis of 307 ER-positive breast cancers showed a correlation between total survival and OPG expression, but not with RANK and RANK ligand expression. Moreover, OPG expression appeared to be associated with a lower rate of G3 tumours and an increased rate of PR-positive tumours [19].

However, in view of the limited data it is necessary to interpret the findings of both studies with care. The demonstrated positive effects occurring with higher OPG expression and lower RANK expression with regard to DFS and OS correlate to established prognostic factors such as grading, tumour size or hormone receptor status. Further studies are needed to show whether there is indeed a connection between RANK, RANK ligand, OPG, and the underlying tumour biology.

Therapeutic Approaches

The focus on indications for bone targeted therapy is increasing. One of the most important areas is the therapy of osseous metastases and the reduction of skeletal-related complications (s. also **Table 1**). The adjuvant therapy of breast cancer also includes indications for bone targeted therapy to prevent and treat therapy-induced osteoporosis. This therapy also serves to prevent metas-

tases. But data is also available on primary prevention in healthy patients without breast cancer. The different therapeutic approaches and their mechanisms of action are discussed below.

Bisphosphonates

Bisphosphonates have been part of the standard therapy in the multimodal therapy of breast cancer since many years. Bisphosphonates bind to hydroxylapatite crystals at the surface of the bone and are absorbed by osteoclasts during bone resorption. As soon as a normal osteoclast absorbs the bisphosphonates from the surface of the bone, the osteoclast loses its ruffled border. This results in a disorganisation of the entire cytoskeleton [21]. The intracellular presence of bisphosphonates leads to cell death of the osteoclast through apoptosis [12].

Bisphosphonates do not merely affect bone but also appear to have an anti-tumour effect. Coleman et al. showed in a study in 2010 that the inclusion of bisphosphonates in the neoadjuvant therapy of breast cancer resulted in significantly better tumour regression rates compared to a control group [6]. In this study, the breast cancer patients were given placebo-controlled zoledronate i.v. every 4 weeks in addition to preoperative chemotherapy. At the end of the therapy, patients in the zoledronate group had significantly smaller tumour sizes compared to the placebo group ($p=0.002$) and a significantly higher rate of pathologically complete remission (pCR, $p=0.030$). The percentage of breast-conserving therapies done in this group was 35% and thus almost 3-times higher than in the control group [6].

An anti-tumour effect of bisphosphonates has not only been demonstrated in neoadjuvant therapies but also in adjuvant therapies. In the ABCSG-12 study Grant et al. showed that premenopausal breast cancer patients who receive zoledronate in addition to their adjuvant therapy had a significantly improved DFS [10]. Similar findings were reported by Eidtmann et al. for the ZOFAS study [9]. In the AZURE study which investigated a total of 3360 patients, no significant difference with regard to DFS or OS was found between patients with and those without adjuvant zoledronate therapy after a median follow-up of 59 months. However, a subgroup analysis found a significant improvement in total survival of postmenopausal patients (>5 years after menopause) and of patients older than 60 years after receiving adjuvant zoledronate therapy ($p=0.017$) [5]. The NSABP-B-34 study investigated the adjuvant administration of clodronate in a randomised, placebo-controlled study ($n=3200$ patients). No significant difference to the placebo group with regard to DFS was found after a follow-up of 8.4 years (presented at SABCS 2011). Based on the current data, the administration of clodronate for a period of 2 years and of zoledronate for a period of 3–5 years in postmenopausal breast cancer patients and hormone receptor-positive pre-menopausal breast cancer patients (undergoing endocrine therapy alone) is recommended as an adjuvant therapy (for current recommendations cf. www.ago-online.org).

Bisphosphonates may also have a prophylactic effect on the prevention of metastases and the primary prevention of breast cancer. Diel et al. showed that a 2-year administration of clodronate to patients with positive disseminated tumour cells led to a significantly reduced rate of distant metastases – both bone metastases and visceral metastases – over a period of 3 to 5 years. At follow-up after 9 years this effect was no longer significant, however overall survival (OS) in the clodronate group was still signif-

Table 1 Bisphosphonates and denosumab in the treatment of bone metastases of breast cancer: overview of active agents including information on dosages and mode of application.

Active agent	Dosage	Mode of application
Denosumab	120 mg q4w	s. c.
Zoledronate	4 mg q4w	i. v.
Ibandronate	50 mg/d	p. o.
Ibandronate	6 mg q3w/q4w	i. v.
Clodronate	1 600 mg/d	p. o.
Clodronate	1 500 mg q3w/q4w	i. v.
Pamidronate	90 mg q3w/q4w	i. v.

icantly higher than in the group of patients without bisphosphonates [8].

When considering the prophylactic administration of bisphosphonates, it is important to mention the results of the WHI study. Chlebowski et al. showed in 2010 that patients with no history of breast cancer and oral bisphosphonate therapy had a significantly lower incidence of breast cancer compared to patients who did not take bisphosphonates (HR = 0.68, 95% CI: 0.52–0.89, $p < 0.01$) [4]. The WHI study investigated around 2800 patients, all of whom either took orally administered bisphosphonate alendronate (90% of patients) or etidronate (10% of patients). Average follow-up was 7.8 years. However, it is important to mention that while incidence of invasive breast cancer was significantly reduced in this group, the incidence of ductal carcinoma in situ was significantly increased [4]. When the findings of subgroups were studied and correlated with estrogen receptor (ER) positivity, the lower incidence was particularly significant for ER-positive breast cancers (HR = 0.70, 95% CI: 0.52–0.94, $p = 0.02$), while ER-negative carcinomas only showed a trend towards a lower incidence. An Israeli case control study also showed that women who had been taking bisphosphonates for a period of more than one year had a relative risk reduction of 28% for the development of breast cancer [17]. The reasons for the positive effect of bisphosphonate administration on breast cancer prophylaxis demonstrated in these two studies have not been clinically elucidated yet. However, taking bisphosphonates as breast cancer prophylaxis cannot yet be recommended based on the current data.

Denosumab

In 2011 the European Union approved the use of denosumab as a new therapy option for the treatment of bone metastases in breast cancer. Denosumab is a human monoclonal antibody which binds to RANK ligand [18], which is expressed on osteoblasts and tumour cells and prevents RANK ligand from binding to pre-osteoclasts. The osteoclast precursor cells are prevented from fusing to form active osteoclasts, which inhibits osteolysis. Stopeck et al. showed in patients with metastasised breast cancer that denosumab administration led to a significant reduction of skeletal-related events compared to the administration of zoledronate ($p = 0.01$, HR = 0.82, 95% CI: 0.71–0.95) [23]. However, the study, which had more than 1000 patients per study arm, did not show any difference with regard to overall survival or disease-free survival [23].

Side Effects of Therapy

Overall, both bisphosphonates and denosumab are tolerated very well by patients. Immunological investigations of patients who had taken the monoclonal antibody denosumab for a period of more than 12 months found that it had no effect on the number of leukocytes, T-cells, B-cells or NK-cells [22]. Most side effects took the form of acute phase reactions, most notably after bisphosphonate administration (cf. [Table 2](#)). Side effects primarily consisted of pyrexia, but bone pain and myalgia were also reported. Other side effects included kidney toxicity, with a reported incidence of between 4.9 and 8.5%. Patients were also informed of the possibility of jaw osteonecrosis. The incidence of this in patients undergoing a bisphosphonate therapy, e.g. with

Table 2 Side-effects of bisphosphonate and denosumab therapy on bone metastases of breast cancer (modified according to Stopeck et al. 2010 [23]).

Side effect	Zoledronic acid (n = 1013) n (%)	Denosumab (n = 1020) n (%)
Adverse events (AEs)	985 (97)	977 (96)
Serious adverse events (SAEs)	277 (27,3)	106 (10,4)
Pyrexia	116 (11,5)	9 (0,9)
Bone pain	36 (3,6)	13 (1,3)
Chills	36 (3,6)	3 (0,3)
Arthralgia	32 (3,2)	15 (1,5)
Flu symptoms	23 (2,3)	5 (0,5)
Myalgia	22 (2,2)	7 (0,7)
Kidney toxicity (AEs)	86 (8,5)	50 (4,9)
Kidney toxicity (SAEs)	15 (1,5)	2 (0,2)
Jaw osteonecrosis	14 (1,4)	20 (2,0)

zoledronate, is 1.4% and rates are similarly low in patients receiving denosumab, with a reported incidence of 2.0% [23]. In addition to informing patients about potential side effects, jaw osteonecrosis, and the early symptoms of jaw osteonecrosis, all patients should undergo a dental examination prior to beginning therapy, followed by regular dental check-ups. It is recommended that patients have any necessary extensive dental work done prior to therapy, if possible, to prevent jaw osteonecrosis. Elective dental work with manipulation of the jaw bone should be avoided during bisphosphonate or denosumab therapy [7, 25]. If the patient has a high risk of jaw osteonecrosis, bisphosphonates should be administered orally.

What is the clinical explanation for the incidence of jaw osteonecrosis with bisphosphonate or denosumab therapy? Both bisphosphonates and denosumab are anti-resorptive agents. Administration of these agents results in the inactivation of osteoclasts, either through osteoclast apoptosis or through the inhibition of osteoclastogenesis. This leads to a disruption of the interaction or an imbalance in the relationship between osteoblasts and osteoclasts. Physiological bone remodelling is no longer possible. This can prevent or delay bone healing processes and could be a possible cause of jaw osteonecrosis.

Future Therapy Options

Bisphosphonates and denosumab were initially used in osteoporosis therapy. A look at other new substances used in osteoporosis therapy could be useful.

One agent currently being investigated is odanacatib, a small molecule which inhibits the release of cathepsin K in osteoclasts. This prevents osteoclasts from causing type-1 collagen lysis in bone matrix [16] (cf. [Table 3](#)).

Another agent currently being investigated in phase 1 and phase 2 trials is saracatinib, an Src inhibitor which affects the proton pumps and chloride channels of osteoclasts, and thus influences the acidity of the environment between the osteoclast and the surface of the bone tissue required for bone lysis [16] (cf. [Table 3](#)).

Both substances are currently being investigated in phase I, II, and III trials of osteoporosis patients. A phase III trial of odanacatib with more than 16000 osteoporosis patients is currently underway and is expected to end in 2012. It should be noted that

Table 3 New approaches for the treatment of osteoporosis (selection) including the mode of action.

Active agent	Drug class	Mode of action
Odanacatib	small molecule	<ul style="list-style-type: none"> ▶ inhibits the release of cathepsin K in osteoclasts ▶ osteoclasts cannot cause lysis of type-1 collagen in bone matrix
Saracatinib	Src inhibitor	<ul style="list-style-type: none"> ▶ affects proton pumps and chloride channels of osteoclasts ▶ and thus affects the acidity of the environment between the osteoclast and the surface of the bone tissue required for osteolysis

compared to typical anti-resorptive treatments, the use of either substance does not result in a reduced number, availability or even apoptosis of osteoclasts but only prevents osteolysis by active osteoclasts. Osteoclast-osteoblast communication continues, resulting in a certain amount of physiological bone remodeling. It could well be that the use of these substances will result in fewer side effects such as jaw osteonecrosis [16].

It must be stressed, however, that not all substances which offer promising results for the treatment of osteoporosis will also be beneficial in oncological therapy. Thus, the N-terminal fragment of parathyroid hormone (teriparatide) stimulates osteoblasts, and studies have reported an increase in bone metastases and osteosarcomas [24].

Nevertheless, developments in the field of osteoporosis therapy look exciting and promising and could, in the medium term, also offer benefits to breast cancer patients.

Conflict of Interest



All co-authors have consented to the publication. J. T. Ney holds a consultancy position at Novartis.

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