RANK, RANKL and OPG Expression in Breast Cancer – Influence on Osseous Metastasis

RANK-, RANKL- und OPG-Expression beim Mammakarzinom – Einfluss auf ossäre Metastasierung

Authors J. T. Ney[1], T. Fehm[2], I. Juhasz-Boess[1], E. F. Solomayer[1]

Affiliations
[1] Department of Gynaecology, Obstetrics and Reproductive Medicine, University Hospital of the Saarland, Homburg/Saar
[2] Tübingen University Department of Gynaecology, University Hospital of Tübingen, Tübingen

Key words
- RANKL
- OPG
- breast cancer
- bone metastasis
- denosumab

Schlüsselwörter
- RANKL
- OPG
- Mammakarzinom
- Knochenmetastase
- Denosumab

Abstract

In women, malignant breast tumours are among the most common malignant diseases in Europe. In advanced breast cancer, the risk of bone metastasis increases to 65–75%. The discovery of the physiological bone metabolism parameters RANK (receptor activator of nuclear factor-κB), RANKL (receptor activator of nuclear factor-κB ligand) and OPG (osteoprotegerin) as well as their pathophysiological involvement in bone-related diseases is the subject of new therapeutic strategies. The formation of osteolytic bone metastasis requires increased osteoclast activity. Activation of osteoclasts by excessive direct RANKL or reduced OPG expression of osseous metastatic tumour cells remains to be elucidated. More than 50% of primary breast cancer cells express OPG and RANK, while RANKL could be detected only in 14–60%. Increased OPG concentrations in the serum of patients with bone metastases have been shown in several studies, whereas the RANKL results are described in an opposite manner. The use of OPG as a biomarker for the detection of osteolytic bone metastases is not consistent and needs to be proved in further studies. Increased RANKL activity was found in diseases characterised by excessive bone loss and formed the basis of new therapeutic options. In several studies, a human monoclonal antibody to RANKL (denosumab) was investigated for the treatment of bone diseases. Denosumab is a promising therapeutic option due to its bone-protective effects.

Zusammenfassung

Maligne Erkrankungen der Brust zählen in Europa zu den häufigsten bösartigen Tumoren bei Frauen. Bei fortgeschrittenem Brustkrebs erhöht sich das Risiko einer Knochenmetastase auf 65–75%. Die Entdeckung der physiologischen Knochenstoffwechselparameter RANK (receptor activator of nuclear factor-κB), RANKL (receptor activator of nuclear factor-κB ligand) und OPG (osteoprotegerin) sowie deren pathophysiologische Beteiligung bei ossär bedingten Erkrankungen ist Gegenstand neuer Therapiestrategien. Gerade die Entstehung von osteolytischen Knochenmetastasen setzt eine gesteigerte Osteoklastenaktivität voraus. Eine Aktivierung der Osteoklasten durch übermäßige direkte RANKL- oder reduzierte OPG-Expression ossär metastasierter Tumorzellen ist bis heute nicht eindeutig geklärt. In über 50% der Fälle exprimierten primäre Mammakarzinomzellen OPG und RANK, während RANKL in nur 14–60% nachgewiesen werden konnte. Erhöhte OPG-Konzentrationen im Serum von Patientinnen mit Knochenmetastasen konnten in mehreren Studien gezeigt werden, während die RANKL-Resultate gegensätzlich beschrieben sind. Eine Verwendung von OPG als Biomarker zur Detektion von osteolytischen Knochenmetastasen ist nicht einheitlich und muss durch weitere Studien belegt werden. Eine erhöhte RANKL-Aktivität konnte bei Krankheiten mit ausgedehntem Knochenverlust gefunden werden und bildete die Grundlage neuer Therapieoptionen. In mehreren Studien wurde ein humaner monoklonaler Antikörper gegen RANKL (Denosumab) zur Behandlung ossär bedingter Erkrankungen untersucht. Denosumab stellt wegen seiner knochenprotektiven Wirkung eine vielversprechende Therapieoption dar.

received 19.7.2011
revised 19.12.2011
accepted 21.12.2011

Bibliography
DOI http://dx.doi.org/10.1055/s-0031-1298276
Geburtsh Frauenheilk 2012; 72: 385–391 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0016-5751

Correspondence
Dr. rer. nat. Jasmin Teresa Ney
Department of Gynaecology, Obstetrics and Reproductive Medicine
University Hospital of the Saarland
Kirrberger Straße
66421 Homburg/Saar
jasmin.ney@uks.eu

Introduction

With a proportion of 29% of all tumour-related diseases in Germany, breast cancer is one of the most common malignant conditions in women. The contextual relationship behind breast cancer's increased tendency to form distant metastases in bone is the subject of ongoing research projects aimed at developing new therapeutic options.

This overview paper intends to discuss the direct pathophysiological involvement of breast tumour cells in bone metabolism through the expression of the bone metabolism mediators RANK (receptor activator of nuclear factor κB), RANKL (receptor activator of nuclear factor κB ligand) and OPG (osteoprotegerin), along with their therapeutic applications.

Bone Metabolism

Bony tissue is made up of 65% hydroxyl apatite crystals and around 35% organic matrix. The organic matrix is made up of 90% type 1 collagen and 10% non-collagenous proteins such as fibronectin, osteocalcin, osteopontin and bone sialoprotein [1].

Bone metabolism is a lifelong, dynamic process characterised by interspersing periods of bone resorption and bone formation. Bone cells are involved in the exchange of old bony tissue and the formation of new bony substance. These include osteoblasts, responsible for bone growth, and osteoclasts, responsible for bone resorption. Osteoblasts are formed from pluripotent mesenchymal stem cells, produce the organic base substance (osteoid) and are responsible for the mineralisation of bone. Osteoclasts are formed from haematopoietic stem cells, differentiate into multinucleated giant cells and occur in the bone's absorption zone. The interaction between the bone's stromal cells, the osteoblasts and the osteoclasts is controlled in part by the bone metabolism mediators RANK, RANKL and OPG [1–3].

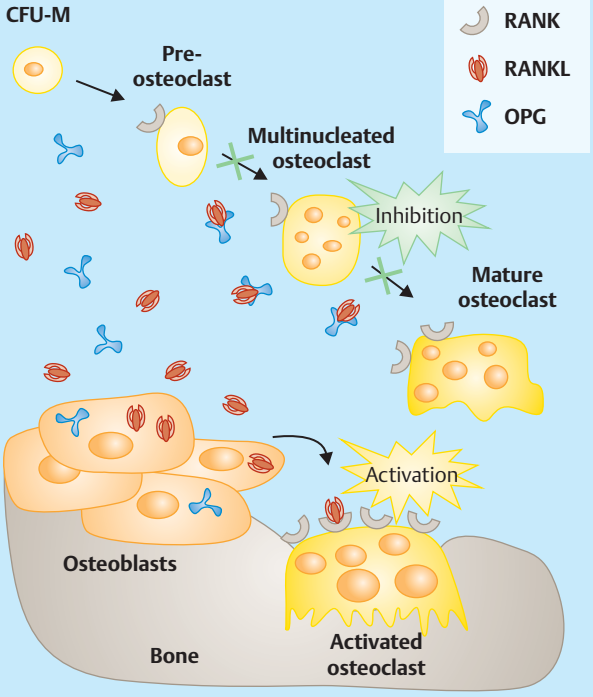

Fig. 1 Control of osteoclast differentiation by the RANK/RANKL/OPG signal pathway. RANKL is formed by osteoblasts and bone marrow stromal cells and binds to the RANK receptor on osteoclast precursor cells (CFU-M: colony forming unit-macrophage) and mature osteoclasts. This interaction promotes the differentiation and activation of osteoclasts. OPG is also formed by bone marrow stromal cells as well as osteoblasts and neutralises RANKL. OPG therefore has an inhibitory effect on osteoclast differentiation and activation. The ratio between RANKL and OPG determines whether bone-forming or bone-removing processes dominate (based on [60]).

Physiological Function of RANK, RANKL and OPG in Bone Metabolism

The discovery of the RANK/RANKL/OPG signal pathway has contributed significantly to the understanding of physiological bone metabolism.

RANKL belongs to the family of TNF (tumour necrosis factor) ligands and is mainly formed by bone marrow stromal cells, osteoblasts and T lymphocytes [4,5]. It is a membrane-bound peptide which can also be converted into a secreted form following post-translational processing by TACE (TNF-α-converting enzyme like protease) [6]. The interaction between RANK and RANKL stimulates the differentiation and fusion of osteoclast precursor cells as well as the activation of mature osteoclasts [5] (**Fig. 1**). RANKL also play a role in the accumulation of osteoclasts on the surface of the bone and in the extension of their life cycle through the inhibition of apoptosis [7,8]. RANK is a homotrimeric membrane protein (616 amino acids) from the TNF receptor family and, in addition to osteoclasts, is also expressed by lymphocytes and dendritic cells [9,10]. RANK and RANKL-deficient mice exhibited reduced or non-existent osteoclast differentiation, severe osteopetrosis and also immunological defects such as missing lymph nodes [11,12]. However rats who over-expressed OPG, in which RANKL is continually inhibited, demonstrated a higher bone density without malformation of the lymphoid organs and with no impairment of the immune response [13]. By contrast, over-expression of RANKL in mice led to a reduced bone density and – as a result of the increased number of osteoclasts – to osteoporosis [14]. The subcutaneous injection of recombinant RANKL in vivo also led to increased bone loss, the increased production of bone absorption markers and a reduction in bone strength [15].

Over-expression of OPG, a member of the TNF receptors family, in vivo leads to increased bone mass and reduces the number and activity of osteoclasts [16]. Independent of this, scientists in Japan have been able to confirm the osteoclast-inhibiting effect of OPG in vitro [17]. Within the bone, OPG is produced by cells from the osteoblast series and secreted as a monomer or homodimer [18]. As the level of differentiation of the cell increases, so does OPG production [19]. OPG acts as a competitive receptor antagonist by binding and neutralising membrane-bound and soluble RANKL. This suppresses the differentiation and fusion of osteoclast precursor cells and inhibits the activation of mature osteoclasts [20,21], (**Fig. 1**). Transgenic mice who over-express OPG also exhibit, similar to RANKL and RANK-deficient mice, a bone-protective effect and osteopetrosis [11,12,16]. The switching off of OPG in vivo, however, leads to massive osteoclast activity and associated osteoporosis [22].

Interaction between Tumour and Bone Cells

In some malignant diseases (multiple myeloma, prostate cancer and breast cancer) with associated bony involvement, impaired regulation of the RANK/RANKL/OPG system plays a significant role.

An invasion of tumour cells into the bony tissue is usually associated with osteolytic lesions (breast cancer [23], multiple myeloma [24]) and, more rarely, with osteoblast metastases (prostate cancer [25]). Interaction between the tumour cells and the bone's micro-environment, based on the principle of the "soil and seed" hypothesis, is crucial for the initiation of osteolytic bone metastasis [26,27]. Tumour cells secrete soluble factors (e.g. cytokines, hormones and growth factors) which ultimately lead to osteoclast activation and thus to bone-destroying processes [28,29]. The PTHrP (parathyroid hormone-related protein) produced by tumours induces the increased expression of RANKL by the osteoblast stromal cells, thereby leading to increased osteoclast activation [30]. Osteoclastic bone absorption releases growth factors such as TGF-β (transforming growth factor β), BMPs (bone morphogenic proteins) or IGF (insulin-like growth factor) from the bone matrix, which in turn contributes towards the increased proliferation of tumour cells. This results in a vicious circle involving the growing proliferation of tumour cells and increased osteolysis [31–33] (**Fig. 2**).

To what extent breast cancer cells are themselves able to express the bone metabolism mediators RANK, RANKL and OPG and, as a result, actively intervene in the metabolism of bone will be summarised in the following section.

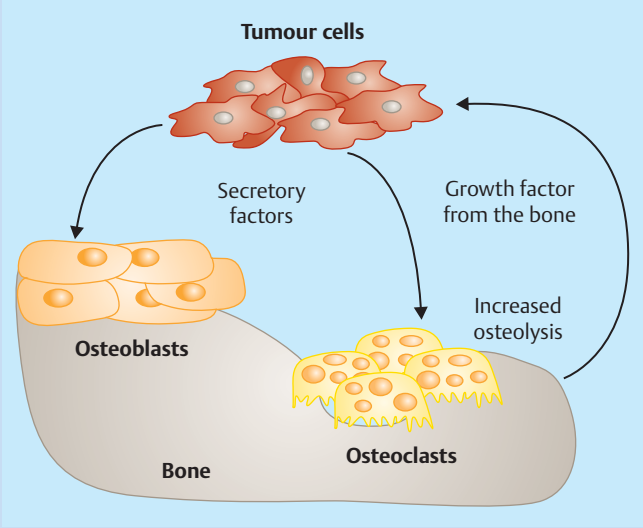

Fig. 2 Interaction between tumour and bone cells. Tumour cells secrete soluble factors that lead directly or indirectly, through the activation of osteoblasts, to increased osteoclast activity and associated bone destruction. Bone resorption leads to the release of growth factors and increased tumour proliferation. A vicious circle arises involving the increasing proliferation of tumour cells and increased osteoclast activation (based on [71]).

RANK, RANKL and OPG Expression in Breast Cancer

Expression analyses at mRNA level

Several studies have analysed the RANK, RANKL and OPG expression of breast cancer cell lines (HCC70, MCF-7, MCF-7 3.1, MCF-7 aro, MDA-MB-231, MDA-MB-435, MDA-MB-453, T47D and ZR 75-1) using RT-PCR analyses. MCF-7, MDA-MB-231 and T47D expressed RANK, and all investigated cell lines, with the exception of MDA-MB-453, expressed OPG, whereas RANKL expression was determined only in HCC70 [30,34,35] (**Table 1**).

A lack of RANKL and different degrees of RANK and OPG expression were also confirmed in primary breast cancer tissue [30,34] (**Table 2**). Using real-time PCR analyses, it was possible to detect a significantly reduced RANKL expression in primary breast cancer cells compared to healthy breast gland tissue, whereas only trends were seen in terms of the expression data in distant metastases and in the OPG expression studies [36] (**Table 2**).

Table 1 RANK, RANKL and OPG expression analysis at mRNA level in breast cancer cell lines.

Cell line	RANK	RANKL	OPG	Reference
HCC70	n. d.	+	+	[35]
MCF-7	+[a]	–	+	[30,35]
MCF-7 3.1	n. d.	–	+	[35]
MCF-7 aro	n. d.	–	+	[35]
MDA-MB-231	+[a]	–	+	[30,34,35]
MDA-MB-435	n. d.	–	+	[35]
MDA-MB-453	n. d.	–	–	[35]
T47D	+[a]	–	+	[30,35]
ZR 75-1	n. d.	–	+	[35]

Note: [a] only analysed by Thomas et al. [30]; n. d.: not determined.

Expression analyses at protein level

The results of a lack of RANKL expression at mRNA level in the MDA-MB-231 [30,34] and MCF-7 cell lines [30] contrast with the results of two further studies. The transmembrane-bound and extra-cellular expression of RANKL in MCF-cells [37] was demonstrated at protein level, as was the co-expression of OPG and RANKL in MDA-MB-231 cells [38]. OPG and RANKL expression in the HCC70 cell line was also confirmed at protein level using immuno-histochemical stainings [35] (**Table 3**).

Table 2 RANK, RANKL and OPG expression analysis at mRNA level of primary/distant metastasised breast tumours and healthy control tissue.

Tissue	RANK	RANKL	OPG	Reference
Primary tumour (n = 12)	100 %[b]	0 %[b]	100 %[b]	[30]
Primary tumour (n = 30)	n. d.	0 %[b]	n. d.	[34]
Primary tumour (n = 24)	n. d.	↓ *[c]	↑ (Trend)[c]	[36]
Liver metastasis (n = 3)	n. d.	↓ (Trend)[c]	↑ *[c]	[36]
Bony metastasis (n = 1)	n. d.	↓ (Trend)[c]	↑ (Trend)[c]	[36]
Ovarian metastasis (n = 1)	n. d.	↓ (Trend)[c]	↓ (Trend)[c]	[36]

Note: [b] qualitative evidence; [c] quantitative evidence (compared with healthy control tissue [n = 18]); * $p < 0.05$; n. d.: not determined.

Table 3 RANK, RANKL and OPG expression analysis at protein level in breast cancer cell lines.

Cell line	RANK	RANKL	OPG	Reference
HCC70	n. d.	+	+	[35]
MCF-7	n. d.	+	n. d.	[37]
MDA-MB-231	n. d.	+	+	[38]

Note: n. d.: not determined.

Several studies have determined the expression of bone metabolism mediators at protein level in human tissue. In the majority of analysed cases, RANK was demonstrated in healthy breast gland tissue (100%) and in primary (65–100%) and osseously metastasised tumour cells (50–100%) [39–41]. In only one study, by Trinkaus et al., RANK expression was not confirmed in healthy tissue and in the primary tumour cells, possibly due to the low case numbers (n = 4) [41] (**Table 4**). It has recently been shown that increased RANK expression in primary breast tumour tissue correlated positively with the development of bone metastasis and is associated with shorter disease-free skeletal survival. Increased RANK expression is also closely associated with negative prognostic parameters (tumour greater than 2 cm; G3; oestrogen receptor-negative tumours). The data was collected at mRNA and protein level [42].

RANKL is expressed heterogeneously in healthy breast gland epithelium and in primary and osseous tumour tissue. In 90–100% of cases, healthy breast gland epithelial cells expressed RANKL, whereas this figure decreased to 14–62.5% in primary breast tumour tissue [39, 43, 44]. One of the studies observed a negative correlation between the RANKL expression of primary tumour cells and the oestrogen receptor status, as well as a positive correlation with the tumour grading [44]. Accordingly, differences in the percentage distribution of RANKL-expressing primary breast cancer tumours, as in the comparison of the van Poznak and Cross study, could relate to the composition of the collective with regard to the oestrogen receptor status. Only Huang et al. were able to observe RANKL expression in all investigated cases (n = 4) in the osseously metastasised tumour cells [45], whereas in the other studies with larger case numbers, osseously metastasised tumour cells expressed RANKL in no more than 2% of the cases [39, 41]. Bhatia et al. also demonstrated a negative correlation between RANKL expression and the osseously metastasising phenotype [39] (**Table 4**).

OPG is not [46] expressed in lobules and glandular tissue with non-neoplastic changes, but was strongly expressed in epithelium showing columnar alteration [43]. Van Poznak et al. and Holen et al. were able to demonstrate OPG expression in primary breast tumour tissue in 40% or 55% of cases, which also correlated positively with oestrogen receptor status [43, 46] and negatively with the ascending tumour grading [46] (**Table 4**).

Intervention in bone metabolism by tumour-related osteoclast activation as a result of direct RANKL expression by osseously metastasised tumour cells cannot be definitively explained due to contrasting study results. In the majority of cases, RANKL expression was lacking in osseously metastasised tumour cells [39, 41, 45]. There is debate over whether or not the existing RANK expression takes priority in osseously metastasised tumour cells due to the lack of RANKL expression. RANK could serve as an anchor and contribute to any possible interaction between tumour cells and bone, or directly activate osteoblasts or stromal cells through interaction with RANKL, which can ultimately result in secondary osteoclast activation and increased osteolysis [39]. The study by Trinkaus et al. also discusses a possible chemotactic stimulus of osteoblast RANKL expression for RANK-expressing, disseminated tumour cells [41]. In vitro data confirms that RANKL can have a contributory effect, depending on its concentration, towards the regulation of the migration of RANK-expressing, healthy epithelial cells and cancer cells [47].

The functional significance of reduced RANKL expression (compared to healthy mammary gland) can only be surmised. OPG is not only able to bind to RANKL as a receptor antagonist but also has a binding affinity to TRAIL (TNF-related apoptosis-inducing ligand). By blockading the TRAIL receptor, TRAIL-induced apoptosis can be inhibited. The lower binding affinity between OPG and TRAIL described in earlier studies [48] has been refuted in a more recent work and is comparable with the affinity between OPG and RANKL [49]. The reduced RANKL expression could lead to an increased interaction between OPG and TRAIL and consequently to a reduced rate of apoptosis [44]. Further studies are needed to clarify the functional significance and the underlying mechanism of reduced RANKL expression.

Table 4 RANK, RANKL and OPG expression analysis at protein level of primary/distant metastasised breast tumours and healthy control tissue.

Tissue	RANK	RANKL	OPG	Reference
Normal tissue (breast) (n = 10)	100%	90%	n. d.	[39]
Primary tumour (n = 58)	100%	61.5%[d] 31.3%[e]	n. d.	[39]
Bony metastasis (n = 43)	100%	2%	n. d.	[39]
Normal tissue (breast) (n = 4)	0%	0%	n. a.	[41]
Primary tumour (n = 4)	0%	0%	n. a.	[41]
Bony metastasis (n = 22)	50%	0%	n. a.	[41]
Normal tissue (breast) (n = 5)	n. d.	100%	100%[f]	[43]
Primary tumour (n = 40)	n. d.	60%	55%	[43]
Primary tumour (n = 400)	n. d.	n. d.	40%	[46]
Primary tumour (n = 400)	n. d.	14%	n. d.	[44]
Primary tumour (n = 14)	65%	n. d.	n. d.	[40]
Bony metastasis (n = 19)	70%	n. d.	n. d.	[40]
Bony metastasis (n = 4)	n. d.	100%	n. d.	[45]

Note: [d] primary tumour tissue from patients without bony metastases; [e] primary tumour tissue from patients with bony metastases; [f] expression only in epithelium showing columnar alteration; n. d.: not determined; n. a.: not analysable.

The following section discusses the extent to which, compared to a healthy test subject, there is a disrupted ratio of soluble OPG and RANKL in the serum of patients with breast cancer and the resulting possible contextual relationships with regard to bone metastasis.

RANKL and OPG Serum Levels in Patients with Breast Cancer

An early study demonstrated that patients with breast cancer had no significant differences in their serum OPG level compared to the healthy control group [50] (**Table 5**).

A comparison of the serum levels of OPG and RANKL in breast cancer patients with osseous involvement and in healthy patients produced conflicting results in two studies. In the study by Mountzios et al., OPG levels and, with the exception of prostate cancer patients, RANKL levels were raised for all tumour types (breast, prostate and lung cancer). A summary of the three tumour types yielded a positive correlation between OPG and the extent of bone metastases [51]. In a recent study by Mercatali et al., on the other hand, significantly lower OPG and RANKL levels were observed in patients with breast cancer and bone metastases. In view of its high specificity (87.7%) and sensitivity (74.1%), the use of OPG as a bio-marker for detecting bone metastases would appear plausible [52] (**Table 5**). One explanation for the contrasting study results could be the different testing methods (ELISA analyses [51], quantitative real-time PCR [52]) used for the determination of RANKL and OPG.

Two further studies confirmed increased serum OPG levels in patients with breast cancer and bony metastases compared to patients with tumours but with no distant metastases. It was, however, not possible to use RANKL or OPG as a bio-marker for detecting bony metastases [53,54] (**Table 5**). One possible explanation discussed for the raised serum OPG level in patients with bony metastases was a secondary reaction aimed at balancing the bone metabolism. Increased OPG production could restrict increasing osteoclast activity in tumour patients with bony metastases [54]. Raised serum OPG levels in patients with post-menopausal osteoporosis, and therefore with associated increased osteoclast activity, were discussed in a similar manner in an earlier work [55].

In a further study, the serum concentrations of OPG and RANKL in breast cancer patients with distant bony metastasis were determined before and after treatment with zoledronate, a bisphosphonate. A trend was observed towards a reduced RANKL/OPG ratio following treatment [56] (**Table 5**).

Further studies are needed to definitively explore any possible clinical use of RANKL and/or OPG as bio-markers. The importance of the RANK/RANKL/OPG system in bone metabolism and the need for increased osteoclast activity for the development of bony metastases, however, open up new therapeutic possibilities.

RANK/RANKL/OPG System – the Basis for Future Treatment Options for Breast Cancer

Initial clinical studies have investigated the effectiveness of recombinant OPG constructs in healthy, post-menopausal women on the treatment of osteoporosis and in patients with malignant diseases on the treatment of tumour-related bone loss. A one-off, subcutaneous administration of Fc-OPG in healthy, post-menopausal women resulted in a suppression of bone absorption markers [57]. In a phase I study, a second construct (OPG-Fc) was tested on patients with multiple myeloma as well as patients with breast cancer and osteolytic bone lesions. In a similar way to the control group treated with bisphosphonate (pamidronate), the one-off administration led to a rapid drop in the bone absorption marker [58]. Risks associated with the use of OPG constructs included the possible generation of neutralising, anti-OPG antibodies and the associated neutralisation of the endogenous OPGs, as well as potential binding to TRAIL and consequently interference with natural tumour defence mechanisms [59]. With the development of the monoclonal human RANKL antibody (denosumab), no further studies involving recombinant OPG constructs were carried out in view of denosumab's high specificity and longer half-life [60]. Several studies demonstrated the bone-protecting effect of denosumab in patients with post-menopausal osteoporosis [61,62] and in patients with rheumatoid arthritis [63,64].

The results of a study investigating the possible use of denosumab in patients with breast cancer with bony involvement are promising. Treatment with denosumab led to a reduced excretion of the bone absorption marker uNTX (urinary N-telopeptide). The reduction in uNTX was of a similar magnitude to that seen in the control group treated with pamidronate but was effective for longer [65]. Supplementary phase II studies confirmed a similar level of suppression of bone turnover in patients with breast cancer and bony involvement treated with denosumab and in those who were given intravenous bisphosphonate. The risk of skeletal complications was also reduced [66,67]. In patients who had high uNTX levels despite bisphosphonate therapy, switching to denosumab resulted in a greater drop in uNTX levels than in patients whose treatment was continued with bisphos-

Table 5 Serum RANKL and OPG level in patients with breast cancer compared with the control group.

Patients	Control	RANKL	OPG	Reference
Breast cancer	Healthy	n. d.	n. s.	[50]
Breast cancer (bony metastasis)	Healthy	↑ *	↑ *	[51]
Breast cancer (bony metastasis)	Healthy	↓ **	↓ **	[52]
Breast cancer (bony metastasis)	Breast cancer (no bony metastasis)	n. s.	↑ *	[53]
Breast cancer[g] (bony metastasis)	Breast cancer[g] (no bony metastasis)	n. d.	↑ *	[54]
Breast cancer (bony metastasis) before BP therapy	Breast cancer (bony metastasis) after BP therapy	n. s.[h]	n. s.	[56]

Note: [g] on Anastrazole therapy; [h] trend towards a reduced RANKL/OPG ratio after treatment; * $p < 0.05$; ** $p < 0.001$; n. d.: not determined; n. s.: not significant; BP: bisphosphonate.

phonates [68]. In a recent clinical phase III study, patients with confirmed breast cancer and at least one bony metastasis were treated with either denosumab (120 mg s.c. and placebo i.v.) or zoledronate (4 mg i.v. and placebo s.c.) and the results compared. In the patients treated with denosumab, suppression of bone turnover was confirmed and skeletal complications were observed to develop more slowly [69].

A further clinical phase III study showed that even patients with breast cancer without bony metastases but with reduced bone density (caused by treatment with aromatase inhibitors) were able to benefit from treatment with denosumab. The treatment significantly increased bone density after 12 or 24 months compared with the control group treated with the placebo [70].

Denosumab, in view of its high specificity, long half-life and good tolerability, represents a highly promising treatment option for bone diseases.

Summary for Implementation in Practice

▼

The discovery of the pathophysiological involvement of the bone metabolism parameters RANK, RANKL and OPG in bone-related malignant diseases has formed the subject of new treatment options. The ratio of RANKL to OPG controls bone-forming and bone-removing processes that are partly responsible for the development of bony metastases.

Contrasting or as yet unreported analyses mean that it has not yet been possible to definitively explain whether an excessive or insufficient expression of RANKL and OPG by breast tumour cells has a direct impact on the physiological ratio of RANKL/OPG. However, several studies have shown that a blockade of RANKL by monoclonal antibodies (denosumab) exhibits a bone-protective effect and represents a highly promising therapeutic option for the treatment of bone-related diseases (e.g. bony metastases, rheumatoid arthritis, post-menopausal osteoporosis).

Conflict of Interest

▼

J.T. Ney holds a consultancy position at Novartis.

References

1 *Keck AV, Pecherstorfer M.* Knochenstoffwechsel bei malignen Erkrankungen. J Miner Stoffwechs 2003; 10: 6–11
2 *Raggatt LJ, Partridge NC.* Cellular and molecular mechanisms of bone remodeling. J Biol Chem 2010; 285: 25 103–25 108
3 *Teitelbaum SL, Ross FP.* Genetic regulation of osteoclast development and function. Nat Rev Genet 2003; 4: 638–649
4 *Kong YY, Feige U, Sarosi I et al.* Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999; 402: 304–309
5 *Lacey DL, Timms E, Tan HL et al.* Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93: 165–176
6 *Lum L, Wong BR, Josien R et al.* Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. J Biol Chem 1999; 274: 13 613–13 618
7 *Fuller K, Wong B, Fox S et al.* TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med 1998; 188: 997–1001
8 *O'Brien EA, Williams JH, Marshall MJ.* Osteoprotegerin ligand regulates osteoclast adherence to the bone surface in mouse calvaria. Biochem Biophys Res Commun 2000; 274: 281–290
9 *Anderson DM, Maraskovsky E, Billingsley WL et al.* A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997; 390: 175–179
10 *Hsu H, Lacey DL, Dunstan CR et al.* Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 1999; 96: 3540–3545
11 *Dougall WC, Glaccum M, Charrier K et al.* RANK is essential for osteoclast and lymph node development. Genes Dev 1999; 13: 2412–2424
12 *Kong YY, Yoshida H, Sarosi I et al.* OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999; 397: 315–323
13 *Stolina M, Dwyer D, Ominsky MS et al.* Continuous RANKL inhibition in osteoprotegerin transgenic mice and rats suppresses bone resorption without impairing lymphorganogenesis or functional immune responses. J Immunol 2007; 179: 7497–7505
14 *Mizuno A, Kanno T, Hoshi M et al.* Transgenic mice overexpressing soluble osteoclast differentiation factor (sODF) exhibit severe osteoporosis. J Bone Miner Metab 2002; 20: 337–344
15 *Lloyd SA, Yuan YY, Kostenuik PJ et al.* Soluble RANKL induces high bone turnover and decreases bone volume, density, and strength in mice. Calcif Tissue Int 2008; 82: 361–372
16 *Simonet WS, Lacey DL, Dunstan CR et al.* Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997; 89: 309–319
17 *Tsuda E, Goto M, Mochizuki S et al.* Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun 1997; 234: 137–142
18 *Yamaguchi K, Kinosaki M, Goto M et al.* Characterization of structural domains of human osteoclastogenesis inhibitory factor. J Biol Chem 1998; 273: 5117–5123
19 *Gori F, Hofbauer LC, Dunstan CR et al.* The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. Endocrinology 2000; 141: 4768–4776
20 *Schneeweis LA, Willard D, Milla ME.* Functional dissection of osteoprotegerin and its interaction with receptor activator of NF-kappaB ligand. J Biol Chem 2005; 280: 41155–41164
21 *Shalhoub V, Faust J, Boyle WJ et al.* Osteoprotegerin and osteoprotegerin ligand effects on osteoclast formation from human peripheral blood mononuclear cell precursors. J Cell Biochem 1999; 72: 251–261
22 *Bucay N, Sarosi I, Dunstan CR et al.* osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998; 12: 1260–1268
23 *Quattrocchi CC, Piciucchi S, Sammarra M et al.* Bone metastases in breast cancer: higher prevalence of osteosclerotic lesions. Radiol Med 2007; 112: 1049–1059
24 *Tian E, Zhan F, Walker R et al.* The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med 2003; 349: 2483–2494
25 *Charhon SA, Chapuy MC, Delvin EE et al.* Histomorphometric analysis of sclerotic bone metastases from prostatic carcinoma special reference to osteomalacia. Cancer 1983; 51: 918–924
26 *Clines GA, Guise TA.* Hypercalcaemia of malignancy and basic research on mechanisms responsible for osteolytic and osteoblastic metastasis to bone. Endocr Relat Cancer 2005; 12: 549–583
27 *Goltzman D.* Osteolysis and cancer. J Clin Invest 2001; 107: 1219–1220
28 *Bendre MS, Margulies AG, Walser B et al.* Tumor-derived interleukin-8 stimulates osteolysis independent of the receptor activator of nuclear factor-kappaB ligand pathway. Cancer Res 2005; 65: 11001–11009
29 *Perez M, Migliaccio S, Taranta A et al.* Melanoma cells stimulate osteoclastogenesis, c-Src expression and osteoblast cytokines. Eur J Cancer 2001; 37: 629–640
30 *Thomas RJ, Guise TA, Yin JJ et al.* Breast cancer cells interact with osteoblasts to support osteoclast formation. Endocrinology 1999; 140: 4451–4458
31 *Blum B, Moseley J, Miller L et al.* Measurement of bone morphogenetic proteins and other growth factors in demineralized bone matrix. Orthopedics 2004; 27: s161–s165
32 *Guise TA, Chirgwin JM.* Transforming growth factor-beta in osteolytic breast cancer bone metastases. Clin Orthop Relat Res 2003; S32–S38
33 *Mundy GR.* Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2002; 2: 584–593

34 *Park HR, Min SK, Cho HD et al.* Expression of osteoprotegerin and RANK ligand in breast cancer bone metastasis. J Korean Med Sci 2003; 18: 541–546
35 *Schubert A, Schulz H, Emons G et al.* Expression of osteoprotegerin and receptor activator of nuclear factor-kappaB ligand (RANKL) in HCC70 breast cancer cells and effects of treatment with gonadotropin-releasing hormone on RANKL expression. Gynecol Endocrinol 2008; 24: 331–338
36 *Reinholz MM, Iturria SJ, Ingle JN et al.* Differential gene expression of TGF-beta family members and osteopontin in breast tumor tissue: analysis by real-time quantitative PCR. Breast Cancer Res Treat 2002; 74: 255–269
37 *Nicolin V, Bortul R, Bareggi R et al.* Breast adenocarcinoma MCF-7 cell line induces spontaneous osteoclastogenesis via a RANK-ligand-dependent pathway. Acta Histochem 2008; 110: 388–396
38 *Nicolin V, Narducci P.* Soluble TRAIL could enhance bone destruction acting on Rank-ligand in estrogen-independent human breast cancer cell line MDA-MB-231. Acta Histochem 2010; 112: 189–192
39 *Bhatia P, Sanders MM, Hansen MF.* Expression of receptor activator of nuclear factor-kappaB is inversely correlated with metastatic phenotype in breast carcinoma. Clin Cancer Res 2005; 11: 162–165
40 *Santini D, Perrone G, Roato I et al.* Expression pattern of receptor activator of NFkappaB (RANK) in a series of primary solid tumors and related bone metastases. J Cell Physiol 2011; 226: 780–784
41 *Trinkaus M, Ooi WS, Amir E et al.* Examination of the mechanisms of osteolysis in patients with metastatic breast cancer. Oncol Rep 2009; 21: 1153–1159
42 *Santini D, Schiavon G, Vincenzi B et al.* Receptor activator of NF-kB (RANK) expression in primary tumors associates with bone metastasis occurrence in breast cancer patients. PLoS One 2011; 6: e19234
43 *Van Poznak C, Cross SS, Saggese M et al.* Expression of osteoprotegerin (OPG), TNF related apoptosis inducing ligand (TRAIL), and receptor activator of nuclear factor kappaB ligand (RANKL) in human breast tumours. J Clin Pathol 2006; 59: 56–63
44 *Cross SS, Harrison RF, Balasubramanian SP et al.* Expression of receptor activator of nuclear factor kappabeta ligand (RANKL) and tumour necrosis factor related, apoptosis inducing ligand (TRAIL) in breast cancer, and their relations with osteoprotegerin, oestrogen receptor, and clinicopathological variables. J Clin Pathol 2006; 59: 716–720
45 *Huang L, Cheng YY, Chow LT et al.* Tumour cells produce receptor activator of NF-kappaB ligand (RANKL) in skeletal metastases. J Clin Pathol 2002; 55: 877–878
46 *Holen I, Cross SS, Neville-Webbe HL et al.* Osteoprotegerin (OPG) expression by breast cancer cells in vitro and breast tumours in vivo – a role in tumour cell survival? Breast Cancer Res Treat 2005; 92: 207–215
47 *Jones DH, Nakashima T, Sanchez OH et al.* Regulation of cancer cell migration and bone metastasis by RANKL. Nature 2006; 440: 692–696
48 *Emery JG, McDonnell P, Burke MB et al.* Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J Biol Chem 1998; 273: 14363–14367
49 *Vitovski S, Phillips JS, Sayers J et al.* Investigating the interaction between osteoprotegerin and receptor activator of NF-kappaB or tumor necrosis factor-related apoptosis-inducing ligand: evidence for a pivotal role for osteoprotegerin in regulating two distinct pathways. J Biol Chem 2007; 282: 31601–31609
50 *Lipton A, Ali SM, Leitzel K et al.* Serum osteoprotegerin levels in healthy controls and cancer patients. Clin Cancer Res 2002; 8: 2306–2310
51 *Mountzios G, Dimopoulos MA, Bamias A et al.* Abnormal bone remodeling process is due to an imbalance in the receptor activator of nuclear factor-kappaB ligand (RANKL)/osteoprotegerin (OPG) axis in patients with solid tumors metastatic to the skeleton. Acta Oncol 2007; 46: 221–229
52 *Mercatali L, Ibrahim T, Sacanna E et al.* Bone metastases detection by circulating biomarkers: OPG and RANK-L. Int J Oncol 2011; 39: 255–261
53 *Leeming DJ, Koizumi M, Byrjalsen I et al.* The relative use of eight collagenous and noncollagenous markers for diagnosis of skeletal metastases in breast, prostate, or lung cancer patients. Cancer Epidemiol Biomarkers Prev 2006; 15: 32–38
54 *Martinetti A, Bajetta E, Ferrari L et al.* Osteoprotegerin and osteopontin serum values in postmenopausal advanced breast cancer patients treated with anastrozole. Endocr Relat Cancer 2004; 11: 771–779
55 *Yano K, Tsuda E, Washida N et al.* Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 1999; 14: 518–527
56 *Mountzios G, Terpos E, Syrigos K et al.* Markers of bone remodeling and skeletal morbidity in patients with solid tumors metastatic to the skeleton receiving the biphosphonate zoledronic acid. Transl Res 2010; 155: 247–255
57 *Bekker PJ, Holloway D, Nakanishi A et al.* The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res 2001; 16: 348–360
58 *Body JJ, Greipp P, Coleman RE et al.* A phase I study of AMGN-0007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. Cancer 2003; 97: 887–892
59 *Bekker PJ, Holloway DL, Rasmussen AS et al.* A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. 2004. J Bone Miner Res 2005; 20: 2275–2282
60 *Kostenuik PJ.* Osteoprotegerin and RANKL regulate bone resorption, density, geometry and strength. Curr Opin Pharmacol 2005; 5: 618–625
61 *Eastell R, Christiansen C, Grauer A et al.* Effects of denosumab on bone turnover markers in postmenopausal osteoporosis. J Bone Miner Res 2011; 26: 530–537
62 *McClung MR, Lewiecki EM, Cohen SB et al.* Denosumab in postmenopausal women with low bone mineral density. N Engl J Med 2006; 354: 821–831
63 *Deodhar A, Dore RK, Mandel D et al.* Denosumab-mediated increase in hand bone mineral density associated with decreased progression of bone erosion in rheumatoid arthritis patients. Arthritis Care Res (Hoboken) 2010; 62: 569–574
64 *Dore RK, Cohen SB, Lane NE et al.* Effects of denosumab on bone mineral density and bone turnover in patients with rheumatoid arthritis receiving concurrent glucocorticoids or bisphosphonates. Ann Rheum Dis 2010; 69: 872–875
65 *Body JJ, Facon T, Coleman RE et al.* A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. Clin Cancer Res 2006; 12: 1221–1228
66 *Lipton A, Steger GG, Figueroa J et al.* Randomized active-controlled phase II study of denosumab efficacy and safety in patients with breast cancer-related bone metastases. J Clin Oncol 2007; 25: 4431–4437
67 *Lipton A, Steger GG, Figueroa J et al.* Extended efficacy and safety of denosumab in breast cancer patients with bone metastases not receiving prior bisphosphonate therapy. Clin Cancer Res 2008; 14: 6690–6696
68 *Fizazi K, Lipton A, Mariette X et al.* Randomized phase II trial of denosumab in patients with bone metastases from prostate cancer, breast cancer, or other neoplasms after intravenous bisphosphonates. J Clin Oncol 2009; 27: 1564–1571
69 *Stopeck AT, Lipton A, Body JJ et al.* Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol 2010; 28: 5132–5139
70 *Ellis GK, Bone HG, Chlebowski R et al.* Effect of denosumab on bone mineral density in women receiving adjuvant aromatase inhibitors for non-metastatic breast cancer: subgroup analyses of a phase 3 study. Breast Cancer Res Treat 2009; 118: 81–87
71 *Sterling JA, Edwards JR, Martin TJ et al.* Advances in the biology of bone metastasis: how the skeleton affects tumor behavior. Bone 2010; 48: 6–15

Deutschsprachige Zusatzinformationen online abrufbar unter:
www.thieme-connect.de/ejournals/toc/gebfra.