OPG/RANKL/RANK Pathway as a Therapeutic Target in Cancer

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Abstract: Bone metastases play an important role in the morbidity and mortality of patients with malignant disease. Despite therapeutic advances in the treatment of solid organ malignancy such as lung cancer, less development on metastasis interventions has been forthcoming. More recent research has focused on molecular pathway manipulation in the prevention and treatment of metastatic bone disease and associated complications such as bone pain and hypercalcemia. The osteoprotegerin/receptor activator of nuclear factor-κβ ligand/receptor activator of nuclear factor-κβ pathway, which is physiologically involved in bone turnover, has been of considerable interest, and recent promising data have been revealed. In this study, we describe this molecular pathway in terms of its natural physiological function, manipulation for therapeutic benefit, and recent clinical trial results.

Key Words: Osteoprotegerin (OPG), Receptor activator of nuclear factor-κβ ligand (RANKL), Receptor activator of nuclear factor-κβ (RANK) cancer, Metastasis, Bone disease, Clinical trials.

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More than 1.5 million patients with cancer worldwide experience bone metastases that are most commonly associated with cancers of the prostate, breast, and lung, where, in particular, 30 to 40% and 50% of patients with lung cancer develop bone metastases at some point or have bone involvement at the time of diagnosis, respectively.[1] Furthermore, postmortem studies have shown that approximately 75% of patients with these cancers have bone metastasis at death[2–4] and approximately therapies directed at this process have potential to offer huge benefit in terms of morbidity, hospital admissions, and financial cost and may even improve survival in this large group of patients. One area of continued advancement in the prevention and treatment of bone metastases is the osteoprotegerin (OPG)/receptor activator of nuclear factor-κβ ligand (RANKL)/receptor activator of nuclear factor-κβ (RANK) pathway.

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Since the discovery of these members of the tumor necrosis factor (TNF) and TNF receptor superfamily approximately a decade ago, OPG, RANKL, and RANK have all been found as a unit to have a key role in osteoclast function and thereby bone turnover.[5,6] They are specifically involved in osteoclastic proliferation, differentiation, activation, and apoptosis and have also been implicated in lactation, tumor cell proliferation, and dendritic cell maturation.[6,7]

RANK is a type I homotrimeric transmembrane protein which shows high homology with CD40 and is not only expressed widely on osteoclasts, T and B cells, fibroblasts, dendritic cells, and mammary gland but also on cancer cells including breast and prostate.[8–11] It has an important role in bone turnover highlighted by the fact that RANK knockout mice are profoundly osteopetrotic due to an absence of osteoclasts.

RANKL is a potent osteoclastogenic factor expressed as a type II homotrimeric transmembrane protein on osteoblasts, osteocytes, and marrow stromal cells, which has 30% homology to TNF-related apoptosis inducing ligand (TRAIL) and 20% homology to fas ligand.[10,12,13] It interacts with RANK on the osteoclast membrane and/or OPG, a soluble decoy receptor. It is also secreted as a soluble molecule by activated T lymphocytes.[14] It was originally described by four separate research groups. Two of the groups believed RANKL's important role lay in the immune system, whereas the other two groups believed RANKL's importance was in osteoclastgenesis.[5] RANKL knockout mice have severe osteopetrosis with a complete absence of osteoclasts. They also have defects in early differentiation of T and B cells, lack lymph nodes, and have defects in mammary gland development highlighting "crosstalk" between the immune and skeletal systems.[5,8,14]

OPG is a secreted protein lacking a transmembrane domain produced by many cell types including bone marrow stromal cells and osteoblasts, which blocks osteoclast precursor differentiation by binding RANKL (thus preventing RANK activation).[6] OPG's role as a bone protector has been demonstrated not least by the fact that OPG knockout mice have osteoporosis.[15,16] OPG is also a soluble decoy receptor for TRAIL.[17,18] In binding TRAIL, it prevents this ligand from inducing apoptosis of malignant or transformed cells ultimately enhancing tumor cell survival.[18]

In humans, mutations in all three members of this pathway have been discovered. Three RANKL mutations (V277WfX5, M199K, and dell45–177AA) have been described in individuals with autosomal recessive osteopetrosis

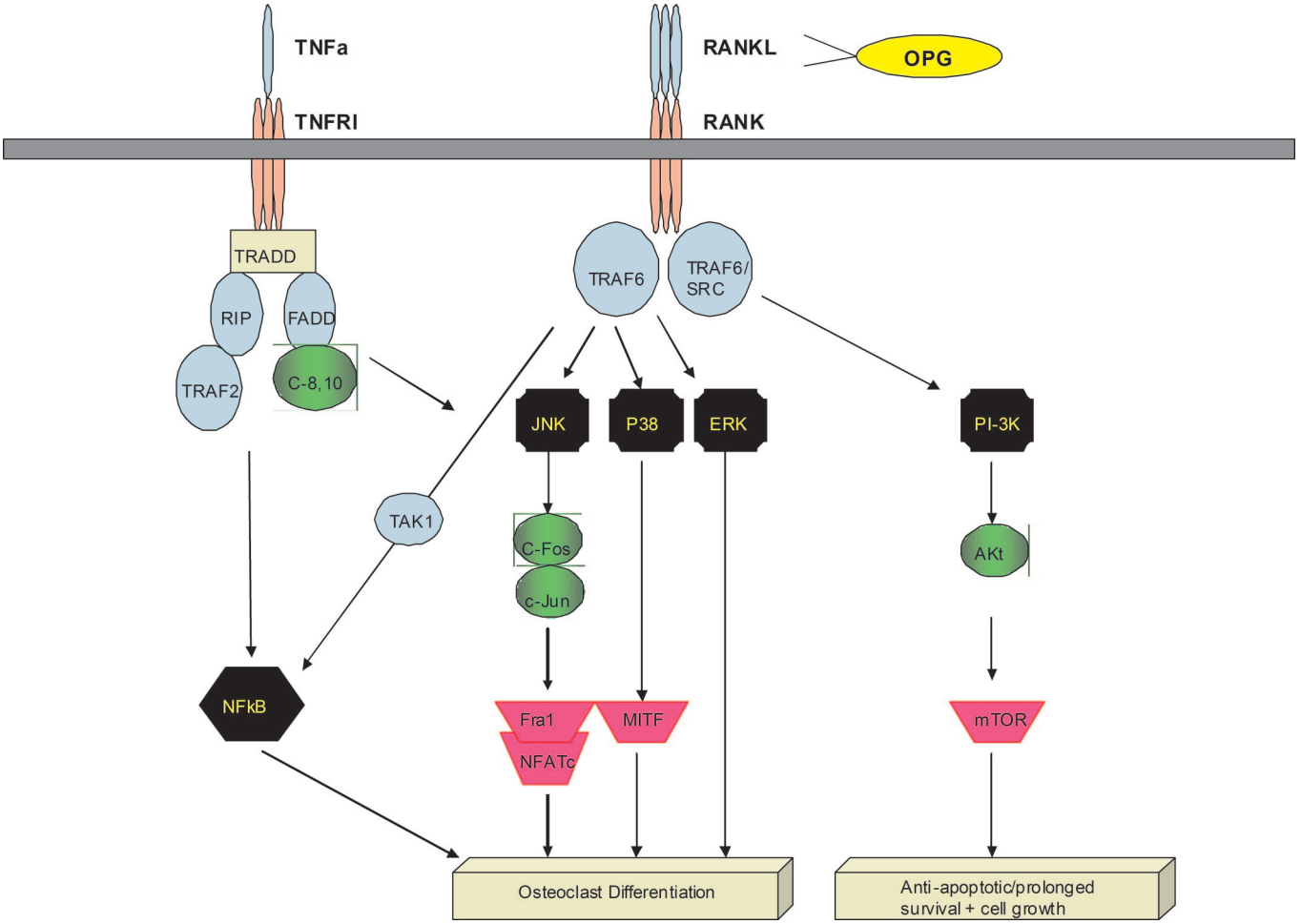

FIGURE 1. The OPG/RANKL/RANK pathway as a therapeutic target. OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κβ ligand; RANK, receptor activator of nuclear factor-κβ.

and osteoclast absence.[5,19,20] Seven RANK mutations (G53R, R129C, R170G, C175R, G280X, W434X, and A244S) have been described in patients with osteoclast deficient osteopetrosis.[5,20,21] Several OPG mutations have been described in clinical reports where genetic deletion or abnormality is associated with juvenile Paget's disease and idiopathic hyperphosphatasia.[22,23]

After the critical role of OPG/RANKL/RANK was highlighted, for example, in their respective knockout mice, research soon focused on this pathway in bone disease. Many factors that were already known to influence bone turnover, such as parathyroid hormone-related protein, Vitamin D3, TNF-a, interleukin-1b, prostaglandin E_2, and macrophage colony stimulating factor, have subsequently been found to up-regulate/down-regulate one or more members of this pathway.[5,24]

Once RANKL binds to RANK, a host of reactions follow which promote the survival, differentiation, and activation of mature osteoclasts.[6] Importantly, as RANK is also expressed on tumor cells, it facilitates similar tumor cell responses.[25]

The binding of RANKL to RANK on the cell membrane leads to receptor trimerization (Figure 1). This results in the recruitment of adaptor molecules such as tumor necrosis factor receptor-associated factor (TRAF)-6 which stimulates the mitogen-activated protein kinase pathways (c-Jun N-terminal kinase [JNK1], extracellular signal regulated kinase 1, and p38) and the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) pathway through tissue growth factor-β-activated kinase. Stimulation of JNK1 results in the mobilization and activation of osteoclastogenic transcription factors such as c-Fos, fos-related antigen-1, and nuclear factor-activated T-cell c1.[26] Stimulation of both the NF-κB and JNK pathways are crucial for monocytic precursor differentiation into osteoclasts.[26] RANK signaling of NF-κB and JNK pathways is synergistically enhanced by the binding of TNF-a to tumor necrosis factor receptor-1 which is facilitated through receptor-interacting protein 1-IκB kinase and TRAF2-GCK complex activation, respectively.[26] TRAF6 also interacts with c-Src stimulating the phosphoinositide 3-kinase-Akt pathway thus influencing osteoclast cytoskeletal changes and survival.[27]

The interaction of RANKL with OPG and their respective concentrations has a direct relationship with the levels of

bone resorption, and in the case of cancer, growth of bone metastasis.[6] Furthermore, RANKL-stimulated osteoclastic bone resorption leads to the release of growth factors such as tissue growth factor-β and platelet-derived growth factor, which in turn stimulate further tumor cell growth through a positive feedback mechanism.[6] RANKL is also up-regulated through parathyroid hormone-related protein, which is released with hormones, chemokines, cytokines, and growth factors by the tumor cells themselves.[6] In contrast, T-cell-produced RANKL can also stimulate osteoclasts to express interferon-β through c-Fos, negatively regulating osteoclast formation.[28]

The OPG/RANKL/RANK system has been widely studied in multiple myeloma-related bone disease where osteolytic bone lesions are associated with pain, hypercalcemia, and pathological fractures.[6] An imbalance in the RANKL/OPG ratio favoring osteolysis has been demonstrated.[6] Moreover, it has become clear that the RANKL/OPG ratio is a key determinant of bone mass in a variety of disorders.[29,30]

RANKL has also been implicated in skeletal metastasis secondary to solid organ tumors such as lung, breast, and prostate. The osteolytic lesions and hypercalcemia are associated with excessive osteoclast activation mediated by RANKL-RANK interactions.[6] Moreover, Huang et al.[31] demonstrated RANKL mRNA and protein in 90% of skeletal lesions in patients with metastasis from lung, breast, thyroid, and prostate cancer. OPG levels and OPG/RANKL ratios have been measured in various cancers and their levels correlate with the degree of bone metastasis.[32,33]

The findings of the research into RANKL and its pathway has led to the emergence of a hypothesis which suggests that the RANKL/RANK system is a prominent "soil" factor in promoting metastasis to bone which is highlighted by the fact that addition of RANKL to RANK expressing prostate and breast cancer cell lines influences their migration.[5,34] Moreover, Maspin, a known metastasis suppressor of prostate epithelial cells, is inhibited by RANK stimulation.[35] The value of manipulating this pathway in the treatment of metastatic disease has been acknowledged by the extensive research performed over the last decade.

OPG therapy had been shown in mouse models to inhibit osteoclastogenesis and tumor growth, osteolysis in multiple myeloma, bone metastasis in prostate cancer, prostate tumor growth, cancer-induced skeletal destruction, and prevent and reverse malignancy-related hypercalcemia and diminishing advanced bone cancer pain.[36–43]

RANK-Fc has been used in mouse studies as a blocking agent for RANKL and has been shown to diminish tumor burden in prostate cancer and has therapeutic benefit in myeloma.[44–46]

Denosumab is an IgG2 monoclonal antibody with a long half-life which binds RANKL with high affinity. It was developed with the aim of treating bone disease primarily mediated by osteoclast activity such as multiple myeloma, bone metastasis, and cancer treatment-induced bone loss.[47]

It has undergone phase II and III clinical trials for postmenopausal osteoporosis, solid organ bone metastases (including lung), treatment-induced bone loss in prostate cancer, and breast/prostate cancer-related metastases and has shown efficacy in the management of these conditions. It has also been effective in bone loss associated with adjuvant aromatase inhibition for breast cancer.[48–55]

These trials included a randomized double-blind, phase III clinical trial in 2046 women with bone metastases from breast cancer comparing the safety and efficacy of subcutaneous monthly denosumab with monthly intravenous zoledronic acid and found that denosumab was superior to zoledronic acid in delaying time to first on-study skeletal-related event (SRE) (Table 1).[56]

Similar findings have been demonstrated in patients with castration-resistant prostate cancer with established bone metastases in a phase III trial involving 1904 men compariny denosumab with zoledronic acid.[57]

Zoledronic acid (Zometa) is a nitrogenous bisphosphonate that disrupts the HMG-CoA reductase pathway by binding farnesyl diphosphate synthase. This results in the inhibition of protein prenylation and the lipid modification of Ras, Rac, and Rho proteins essential to the osteoclast. In particular, these proteins are key to cell survival, osteoclastogenesis, and cytoskeletal arrangement. Although both the HMG-CoA reductase and OPG/RANK/RANKL pathways are physiologically involved in bone turnover, the OPG/RANK/RANKL pathway is thought to play a more central role in bone metastasis highlighted by preclinical research and in vivo work demonstrating increased expression of the RANK/RANKL proteins and abnormal OPG/RANKL ratios in patients with cancer with bone metastases (unlike in the case of the HMG-CoA pathway), possibly explaining the increased therapeutic benefit/targeting of this system in malignant disease.[5,32–34]

Denosumab also has other important practical advantages over bisphosphonate therapy. It is given every 4 weeks as a 120 mg subcutaneous injection, whereas zoledronic acid is administered every 4 weeks through a 15-minute intravenous infusion, requiring significant resource input that may not be widely available. In addition, adverse events are less frequent with denosumab and importantly unlike zoledronic acid, renal function does not need to be monitored.[56,58]

With regard to lung cancer, a randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in 1776 patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma has yielded some interesting results. This study was made up of approximately 10% myeloma, 40% non-small cell lung cancer, and 50% other solid organ cancers (excluding prostate and breast). Denosumab was noninferior (trend toward superiority) to zoledronic acid in preventing or delaying first on-study SRE in patients with advanced cancer metastatic to bone or myeloma.[58] A subset analysis of the same study, which was presented at the American Society of Clinical Oncology 2010 annual meeting, analyzed the treatment data only in solid-tumor patients with bone metastases (of which

TABLE 1. Current Ongoing Clinical Trials with Denosumab[59,61]

Clinical Trial	Disease	Trial (*n*)	Comparison	Main End Point
NCT00321464 phase III	Advanced breast cancer	2046	Denosumab vs. zoledronic acid	Time to first on-study SRE
NCT00556374 phase III	Nonmetastatic breast cancer on aromatase inhibitor therapy	2800	Denosumab vs. placebo	Time to first fracture
NCT00330759 phase III	Advanced solid organ malignancy (including lung) excluding breast, prostate, and multiple myeloma	1776	Denosumab vs. zoledronic acid	Time to first on-study SRE
NCT00286091 phase III	Hormone resistant prostate cancer without bone metastases	1435	Denosumab vs. placebo	Time to first bone metastasis or death
NCT0089674 phase III	Nonmetastatic prostate cancer with androgen deprivation therapy	1468	Denosumab vs. placebo	% baseline change in bone mass density
NCT00321620 phase III	Hormone-resistant nonmetastatic prostate cancer	1904	Denosumab vs. zoledronic acid	Time to first on-study SRE
NCT00259740 phase II	Relapsed multiple myeloma	96	Denosumab	Treatment response

SRE, skeletal-related event.

non-small cell lung cancer was almost half) and found that denosumab significantly delayed the time to the first and subsequent SREs compared with zoledronic acid.[59] These data support further trials into the use of this agent in solid organ malignancy, particularly lung cancer.

Denosumab has also been investigated in the prevention of metastases, specifically, with a randomized, placebo-controlled multicenter phase III trial comparing the effect of denosumab with placebo on bone metastasis-free survival in men with hormone refractory (castrate-resistant) prostate cancer with rising prostate-specific antigen levels who were free of bone metastasis at enrolment. This study involving 1432 men demonstrated a significantly increased median bone metastasis-free survival of 4.2 months and an improved time to first occurrence of bone metastasis in the treated group.[5,60]

This antibody has recently been approved in the European Union and United States for the treatment of osteoporosis and bone disease (single or multiple metastases) associated with solid organ cancer.[5] Further information on the ongoing phase III trials involving denosumab can be found at www.clinicaltrials.gov. They include three trials in prostate cancer (NCT00321620, NCT00089674, and NCT00286091), two trials in breast cancer (NCT00321464 and NCT00556374), and one trial in solid organ tumors and multiple myeloma (NCT00330759) (Table 1).[59]

Other phase III trials are planned including Study NCT01077154—Denosumab as Adjuvant Treatment for Women With High Risk Early Breast Cancer Receiving Neoadjuvant or Adjuvant Therapy. This randomized phase III trial will study the effect of denosumab to see if it can prevent disease recurrence in the bone or in any other part of the body, when it is given as adjuvant therapy for women with early-stage breast cancer, who are at high risk of disease recurrence. This study is currently recruiting patients.[61]

Pathway manipulation may not just provide benefit in patients with bone metastases. Solid organ cancer and lung metastases may also be directly targeted in the future. Schramek et al.[62] demonstrated that medroxyprogesterone acetate can induce RANKL expression in the mammary gland to an enormous extent. RANKL overexpression leads to an acceleration of mammary gland preneoplasias with increased tumor formation after multiparity or treatment with progestins and carcinogens.[63] Inhibiting RANKL in this scenario may offer direct solid tumor suppression.

Tan et al.[64] have very recently demonstrated that RANK signaling in mammary carcinoma enhances metastasis to lung in mice and therefore targeting the OPG/RANKL/RANK pathway may not just benefit patients with bone metastasis but may have therapeutic benefit in lung metastasis also, although further research is required.

Clearly, our understanding of the OPG/RANKL/RANK pathway and its involvement in bone turnover and bone metastasis has evolved over the past decade, made obvious by the number of phase III clinical trials performed and currently running. More recent developments such as its direct role in cancer cell growth and migration (e.g., to the lung) offer huge therapeutic potential once the process has been further unraveled.

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