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

Emmet E. McGrath, MB, PhD

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.

(*J Thorac Oncol.* 2011;6: 1468–1473)

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.

School of Health and Population Sciences, University of Birmingham, Birmingham, United Kingdom.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Emmet McGrath, MB, PhD, School of Health and Population Sciences, University of Birmingham, Birmingham, United Kingdom. E-mail: e.e.mcgrath@bham.ac.uk

ISSN: 1556-0864/11/0609-1468

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

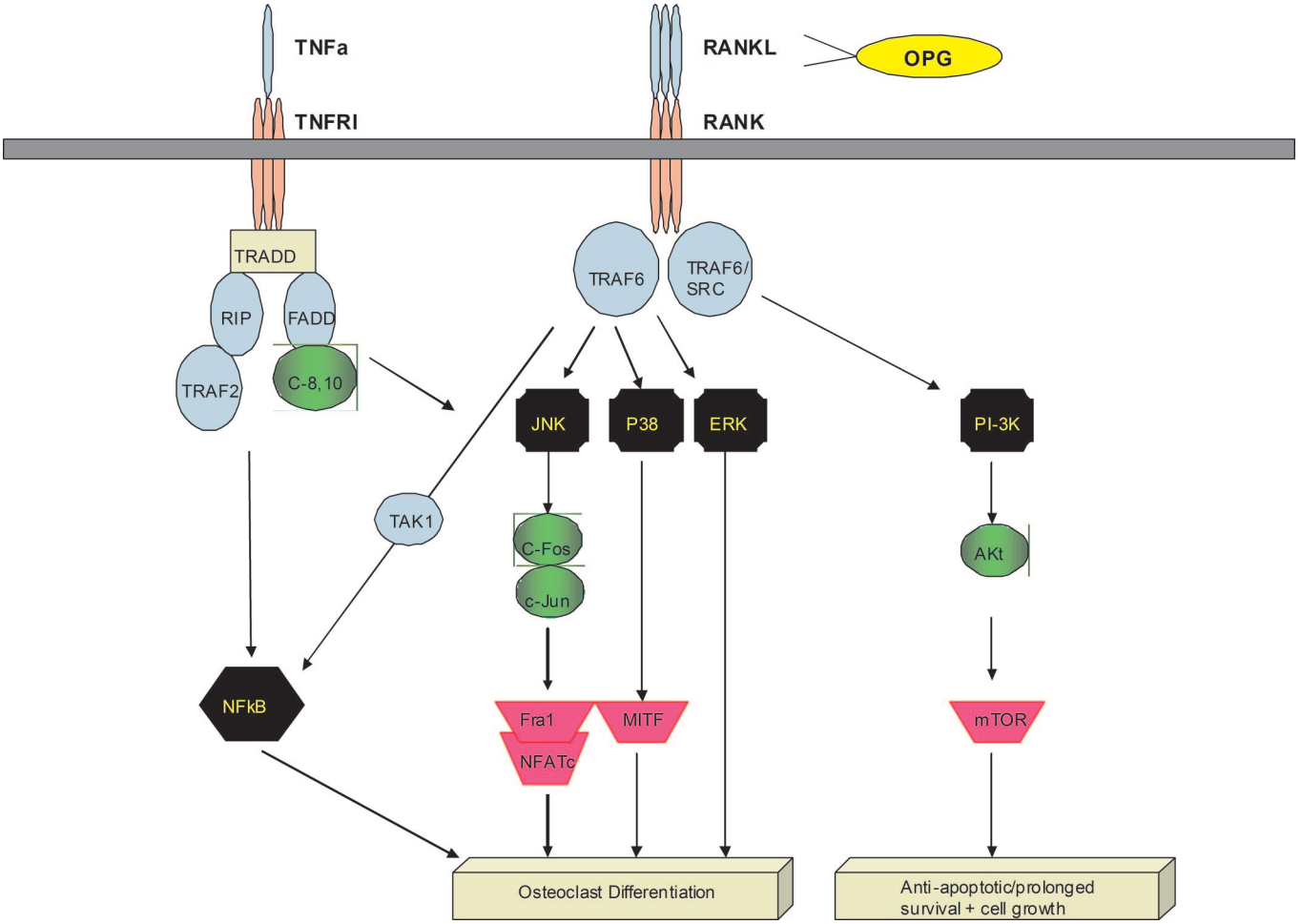

FIGURE 1. The OPG/RANKL/RANK pathway as a therapeutic target. OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κβ ligand; RANK, receptor activator of nuclear factor-κβ.

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

