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RANKL/RANK/osteoprotegerin system as novel therapeutic target in the treatment of primary bone tumors and osteolytic metastases

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ABSTRACT

Primary bone tumors and cancers that metastasize to bone require osteoclastic activity to release tumor-supportive growth factors from bone tissue. A number of systemic and locally acting factors are known to influence osteoclast formation, fusion, activation, and survival. Recently, two critical extracellular regulators of osteoclast differentiation and activation have been identified: receptor activator of nuclear factor (NF-kappaB) ligand (RANKL) and osteoprotegerin (OPG). RANKL is a tumor necrosis factor (TNF)-related cytokine that stimulates osteoclast differentiation from hematopoietic precursor cells and activation of mature osteoclasts. RANKL activates its specific receptor, receptor activator of NF-kappaB (RANK), located on osteoclasts, chondrocytes and dendritic cells. Binding of the RANK ligand to its receptor and osteoclastogenesis are prevented by osteoprotegerin, a decoy receptor produced by osteoblasts and marrow stromal cells. The balance between RANKL and OPG is of major importance in bone homeostasis. Disorders of the RANKL/RANK/OPG system have been linked to several human diseases, including primary bone tumors, skeletal metastases, and hypercalcemia of malignancy. The discovery and characterization of RANKL, RANK and OPG and subsequent studies have changed the concepts of bone metabolism and may form the basis of innovative therapeutic strategies. Novel treatment strategies for bone tumors are emerging based on blockade of the RANKL/RANK interaction. The advantage of these strategies is their potential to selectively target tumor cells. Combining these new strategies with currently available treatments such as chemotherapy and radiation therapy is under investigation, with promising results.

KEY WORDS: *NF-kappa B; Glycoproteins; Bone Neoplasms; Osteoclasts; Neoplasm Metastasis; Bone Resorption*

INTRODUCTION

Bone is a highly hospitable environment for colonization and growth of metastatic tumors (1). Some of the most common human malignancies, notably breast cancer and prostate cancer, have a strong propensity to produce skeletal metastases (2,3). Over 70% of patients with advanced breast or prostate cancer have skeletal metastases (2). Beside secondary tumors, primary tumors such as osteosarcoma and giant-cell tumors can also arise in bone. In order for tumor cells to grow and invade mineralized bone, osteolysis must occur (4,5). Mineralized bone matrix is a rich source of stored growth factors such as transforming growth factor-b (TGF-b), insulin-like growth factors (IGFs) and fibroblast growth factor-2 (FGF-2) (6). Such growth factors, once released from degraded bone matrix, may further accelerate growth of the tumor, which can now expand within the lysed area (4).

The cells responsible for the resorption of bone tissue are the osteoclasts. This cell type is of hematopoietic origin and differentiates in a late phase from the monocyte/macrophage cell lineage to form a giant, multinucleated cell that can attach to mineralized bone tissue (7). An increase in osteolysis is thus usually associated with an increase in the number or the activity of bone-resorbing osteoclasts. A number of systemic and locally acting factors are known to influence osteoclast formation, activation, lifespan, and function (8). These include parathyroid hormone (PTH) (9), PTH-related protein, corticosteroids (10), prostaglandin E_2 (11), hepatocyte growth factor - HGF (12), macrophage inflammatory protein (MIP)-1α and MIP-1β (13), tumor necrosis factor-a (TNF-α) and TNF-β (14), vitamin D, bone morphogenetic protein-2 (15), interleukin-1 (IL-1), IL-6, IL-11 (16). The formation of active osteoclasts requires macrophage colony-stimulating factor (M-CSF) and involves cell-to-cell contact between precursors of the monocyte-macrophage lineage and osteoblasts, marrow stromal cells, and T and B cells (17). Recently, an essential cytokine system for osteoclast biology has been characterized (18,19). This system consists of a ligand, receptor activator of NF-kappaB ligand (RANKL), a cellular receptor, RANK, and a soluble decoy receptor, osteoprotegerin (OPG) (20). Since the discovery of this trio of TNF family proteins, it was fond that nearly all osteotrophic hormones and local pro-resorptive factors produced in the bone microenvironment mediate their action indirectly via RANKL/OPG expression (21).

THE RANKL/RANK/OPG SYSTEM

RANKL is a specific and essential differentiation factor for osteoclast precursors and an essential activation factor for mature osteoclasts. Synonyms for RANKL are OPG ligand, osteoclast differentiation factor (ODF), and TNF-related activation-induced cytokine (TRANCE) (22). The *rankl* gene encodes a TNF superfamily molecule of 316 amino acids, and three RANKL subunits assemble to form the functional trimeric molecule (22). Trimeric RANKL is made as a membrane anchored molecule, but it can be released from the cell surface as a soluble homotrimeric molecules after proteolytic cleavage by the metalloprotease disintegrin TNF-α convertase (TACE) (23). Both soluble and membrane bound RANKL can function as potent agonistic ligands for osteoclastogenesis *in vitro*, but membrane bound RANKL might work more efficiently than soluble RANKL (24). RANKL is extensively expressed by osteoblast/stromal cells (25), primitive mesenchymal cells, chondrocytes, immune and some cancer cells (26). The specific cellular receptor that transduces the actions of RANKL was named RANK. RANK is a member of TNF-R superfamily; it is expressed on the surface of hematopoietic osteoclasts progenitors, mature osteoclasts, chondrocytes, mammary gland epithelial cells and dendritic cells (27). The binding of RANKL to RANK plays an important role in promoting osteoclast differentiation and bone resorption (28). Mice with a genetic mutation of RANK display severe osteopetrosis, cessation of growth, and a defect in tooth eruption (29). Osteoprotegerin (OPG, "protector of bone"), as the name implies, protects bone by potent inhibition of osteoclast activation. It was identified in 1997 by two distinct lines of investigation (30). Synonyms for OPG are osteoclastogenesis-inhibitory factor (OCIF) and TNFR-related molecule 1 (31). Osteoprotegerin is a 401-amino acid secreted glycoprotein with homology to members of the TNF receptor family (27). In contrast to all other TNFR superfamily members, OPG lacks transmembrane and cytoplasmic domains and it is secreted as a soluble protein (17). OPG is produced ubiquitously by many types of cells, and it has very high expression level in the bone marrow microenvironment (32). This glycoprotein acts as a non-signaling decoy receptor for RANKL. Consequently, OPG is an effective inhibitor of osteoclast maturation and activation (24). Mice with overexpression of OPG have decreased osteoclast formation and develop osteopetrosis, whereas mice deficient in OPG have reduced bone mass and develop osteoporosis (33).

The balance between RANKL and OPG is of major importance in bone homeostasis (34). Abnormalities of the RANKL-to-OPG ratio have been implicated in the pathogenesis of postmenopausal osteoporosis, rheumatoid arthritis, periodontal disease, benign and malignant bone tumors, bone metastases, and hypercalcemia of malignancy (35).

THE RANKL-TO-OPG RATIO IN BONE TUMORS

Extensive *in vitro* and animal studies have detected abnormalities of the RANKL:OPG ratio in various benign and malignant neoplasms characterized by abnormal osteoclast function (35). The RANKL:OPG ratio was significantly increased in patients suffering from breast cancer, giant-cell tumors and multiple myeloma (35). Breast cancer cells can secrete PTH-related protein which increases RANKL and decreases OPG expression by osteoblasts, resulting in enhanced osteoclastogenesis and bone resorption with creating cavities in bone where tumor cells are able to expand (36). Giant-cell tumors consist of a stromal cell population of the osteoblastic lineage that overexpresses RANKL, and they also contain large osteoclasts-like "giant cells" that are hyperresponsive to RANKL (37). Together, these effects result in increased osteoclast activity. Myeloma cells increase RANKL expression and inhibit OPG production within the bone microenvironment (38). Furthermore, myeloma cells can bind, internalize, and degrade OPG (34).

Opposite to osteolytic bone tumors, prostate carcinoma skeletal metastases and osteosarcoma often grow as osteoblastic tumors. In these patients high OPG levels have been detected and the RANKL:OPG ratio have been significantly decreased (21). These findings indicate that the RANKL:OPG ratio may affect tumor growth by inhibiting osteoclast activity to allow increased osteoblast proliferation (21). However, some animal studies have been shown that OPG inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone (5). These data suggest that inhibition of osteoclast activity is sufficient to diminish the development of skeletal metastatic prostate tumors that have both osteolytic and osteoblastic components.

UTILIZATION OF THE OPG/RANKL SYSTEM IN THERAPY

It was shown in several studies that in animal disease models administering of OPG could restore the RANKL/OPG imbalance. Beside OPG, a recombinant OPG fusion protein (OPG-Fc) or inhibitory RANK antibodies (RANK-Fc) have been used. RANK-Fc is a recombinant RANKL antagonist that is formed by fusing the extracellular domain of RANK to the Fc portion of human immunoglobulin G_1 (IgG1) (39). Treatment of animals with OPG-Fc or RANK-Fc appears to be effective in conditions associated with upregulated bone resorption (40). Administration of OPG prevents ovariectomy-induced osteoporosis, establishment and progression of osteolytic metastasis, skeletal pain and humoral hypercalcemia of malignancy (35). OPG is currently in phase I clinical trials. In 2001 Bekker et al. tested the effect of OPG in postmenopausal women. In this study, biochemical markers of bone turnover rapidly decreased after a single subcutaneous injection of OPG (41). More recently, a similar approach has been used in patients with myeloma bone disease. In this study, patients receiving 1 mg/kg of OPG-Fc displayed a rapid, sustained decrease of the biochemical marker of bone resorption (42). The results of mentioned experiments suggest that OPG might represent an effective therapeutic option for diseases associated with excessive osteoclast activity (41).

CONCLUSION

RANKL, its receptor RANK, and the decoy receptor OPG are the key regulators for osteoclast development and the activation of mature osteoclasts. The balance of RANKL and OPG determines osteoclast activity (bone resorption). The RANKL:OPG ratio was significantly increased in patients suffering from severe osteolysis. It has become clear that inhibition of RANKL mediated activation of RANK via OPG or a related molecules (recombinant OPG protein or RANK-Fc) may be an effective anabolic treatment for reduced bone mass, management of cancer-induced bone pain, prevention of the development of skeletal metastases, and hypercalcemia of malignancy. The advantage of these therapeutic strategies is their potential to selectively target tumor cells, while exempt normal cells. Combining these new strategies with currently available treatments such as chemotherapy and radiation therapy is under investigation, with promising results.

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