

tor)³ and TRANCE¹⁴, is the obligate ligand for RANK.² It exists naturally in membrane-bound² and secreted forms¹⁸⁻²⁰, and is expressed in stromal fibroblasts, osteoblasts³, T cells², and malignant cells^{5, 20, 21}. Osteoblast expression of RANKL is strongly stimulated by several osteolysis-inducing agents including 1, 25 (OH)₂ vitamin D₃, PTH (and PTHrp), IL-6 and IL-11³. Interaction between RANKL-expressing cells and RANK-expressing pre-osteoclasts triggers an intracellular signaling cascade, which via TNFR associated factors (TRAF) 1-6, effects the NF-κB, serine/threonine kinase Akt/PKB, and protein kinase c-Jun N terminal kinase (JNK) pathways²²⁻²⁷. How these pathways regulate osteoclast activity has yet to be clearly defined.

OSTEOPROTEGERIN (OPG)

OPG, as the name osteoprotegerin implies, protects bone by potent inhibition of osteoclast activation. It is a 401-amino acid secreted glycoprotein. OPG is produced ubiquitously by many types of cells, and has very high expression levels in developing bone. This TNFR super-family member acts as a non-signaling decoy receptor for RANKL. OPG competitively inhibits RANK-RANKL binding, thus blocking osteoclast differentiation. OPG inhibits osteoclastogenesis that is stimulated by 1, 25 (OH)₂ vitamin D₃, PTH, and IL-11^{1, 15, 28}. In mice, under- and over-expression of OPG correlate with clinical osteoporosis and osteopetrosis, respectively^{1, 28-30}.

RANK.Fc

RANK.Fc is a recombinant, soluble form of RANK in which the extracellular domain is expressed as a fusion protein with human IgG Fc. *In vitro* studies have shown that RANK.Fc functions as an antagonist to RANK mediated signaling, and it has been postulated that RANK.Fc acts to sequester endogenously produced RANKL². Transgenic mice expressing RANK.Fc are osteopetrotic, similar to mice with OPG over-expression²².

PRE-CLINICAL STUDIES OF OSTEOPROTEGERIN AND RANK.Fc

CANCER

OPG inhibits osteolysis and decreases tumor burden in an *in vivo* model of skeletal metastasis. Effects of OPG on skeletal metastasis of mouse-derived colonic adenocarcinoma and human-derived breast carcinoma were studied in syngeneic or nude mice, respectively. Treatment resulted in a dose-dependent decrease in radiographically evident lytic bone lesions and histological evidence of decreased skeletal tumor burden and tumor-associated osteoclasts. OPG completely prevented lytic lesions in breast cancer-treated animals⁴.

Although the majority of prostatic carcinoma metastases in bone are clinically osteosclerotic (osteoblastic) rather than overtly osteolytic, OPG inhibits prostate carcinoma growth in bone. Cultured prostatic carcinoma cells produce a soluble form of RANKL, and directly stimulate osteoclastogenesis in the absence of underlying stroma. In mice, OPG treatment completely inhibits osteoclastogenesis and the formation of mixed osteoblastic/osteolytic skeletal tumors²⁰. However, serum OPG levels³¹ and OPG/RANKL mRNA-isolate ratios are elevated

in patients with advanced, metastatic prostate cancer. This seeming contradiction may be explained by the observation that prostate metastases often begin with cycles of osteolysis, which progress to an osteoblastic reaction in later stages. OPG may inhibit the early osteolytic component of prostatic metastasis, thus preventing the destruction of bone requisite for the establishment and neovascularization of metastatic foci. This emphasizes the significance of OPG/RANKL concentration ratios in the development of lytic versus blastic type lesions. Local OPG/RANKL ratios may initially be low, but increase once metastases have been established.

Giant cell tumor (GCT) stromal cells contain high levels of RANKL mRNA, while giant cells themselves exclusively express RANK mRNA. GCT tissues express relatively higher levels of RANKL mRNA in comparison to OPG mRNA *in vitro*. OPG dose-dependently inhibits osteoclast formation and bone resorbing activity in GCT of bone *in vitro*. This process is reversed by treatment with recombinant RANKL. In GCT, osteoclast differentiation can be stimulated *directly* by RANKL expressing tumor stromal cells, as is seen in prostate cancer⁵. This contrasts the proposed indirect induction of osteoclastogenesis seen in breast cancer metastases and multiple myeloma.

Serum OPG levels are lower in patients with multiple myeloma (MM), and correlate inversely with the severity of osteolytic disease³². RANKL and OPG mRNA production are up- and down-regulated, respectively, in cultured bone marrow explants of patients with MM in comparison with healthy controls³³. Myeloma cells stimulate and inhibit RANKL and OPG production, respectively, in co-culture studies³⁴. OPG administration in a mouse model was effective in preventing the formation of osteolytic lesions, and was associated with decreased osteoclast formation and increased bone density³⁵. Osteoclastogenesis was inhibited by the addition of RANK.Fc to co-cultures, and RANK.Fc prevented myeloma-induced bone destruction and slowed disease progression in a SCID-hu murine model of human myeloma³⁴.

OPG blocks, and actually reverses bone cancer pain-related neurochemical reorganization in the spinal cord of mice^{36, 37}. These studies strongly suggest the potential use of OPG in the treatment of bone cancer-related pain.

OPG and RANK.Fc have also been evaluated in humoral hypercalcemia of malignancy. Mice with induced colon-26 cancer have increased PTHrp (parathyroid hormone related-peptide) expression, elevated serum PTHrp, increased bone resorption, and marked hypercalcemia. OPG treatment initiated at the onset of hypercalcemia, or after it occurs, results in inhibition of tumor-induced bone resorption and hypercalcemia, and subsequent normalization of serum ionized-calcium levels⁶.

RANK.Fc effectively inhibits PTHrp-induced resorption in bone cultures *in vitro*. Administration of murine RANK-human Fc fusion protein to normal mice resulted in the disappearance of osteoclasts from the metaphyses of long bones and increased calcification of bony trabeculae as seen radiographically⁷. In

nude mice with subcutaneously implanted human lung cancer tissue, RANK.Fc also inhibits osteoclastic bone resorption and hypercalcemia, without affecting circulating levels of PTHrP. The potential therapeutic value of RANK.Fc is strengthened by its ability to reverse the increase in blood ionized-calcium level even after hypercalcemia had been established in this model⁷.

ARTERIOSCLEROSIS

There is an observed high clinical incidence of arterial calcification in the osteoporotic patient population. While studying osteoporosis in mice, Min et al. examined rates of arterial calcification, demonstrating that OPG treatment was effective in preventing, but not reversing, the high rates of arterial calcification seen in OPG- knockout mice⁸. OPG may affect arterial calcification by decreasing bone resorption and lowering serum calcium levels, but this remains to be proven.

RHEUMATOID ARTHRITIS

Haynes et al. studied the role of OPG in the rheumatoid knee. Cells from human synovial membrane and pannus were cultured and shown to generate osteoclasts capable of forming resorptive lacunae on bone slices. Treatment with recombinant OPG *in vitro* completely inhibited this resorptive process⁹.

PERIPROSTHETIC OSTEOLYSIS

Haynes et al. also performed mRNA analysis of cells derived from periprosthetic tissues containing wear particles³⁸. They showed that osteoclasts formed from periprosthetic tissues both in the presence and absence of osteoblasts. When osteoblasts were not present, RANKL mRNA was expressed in higher rates than OPG mRNA. Their findings suggest that wear debris stimulates macrophage differentiation into osteoclasts via regulation of osteoclastogenic molecules, specifically OPG and RANKL mRNA³⁸.

An *in vitro* mouse study suggests that OPG might be effective in treating wear-induced periprosthetic loosening. Kim et al. were able to demonstrate increased osteoclast formation in a co-culture system in the presence of failed-arthroplasty joint fluid compared with OA joint fluid. They also demonstrated lower OPG levels in the failed-arthroplasty fluid. The increased osteoclastogenesis was blocked by administration of exogenous OPG³⁹.

Childs et al. analyzed effects of RANK.Fc on titanium-induced osteolysis in a mouse calvaria model, making several provocative observations. First, titanium-induced osteoclastogenesis and bone resorption was blocked by intraperitoneal doses of RANK.Fc greater than 1mg/kg, given every 48 hours. A 10mg/kg dose completely inhibited osteolysis. Interestingly, these data were statistically equivalent to titanium implants in RANK-knockout (RANK -/-) mice. Second, mice treated with a single dose 5 days following implantation were depleted of TRAP+ (tartrate-resistant acid phosphatase-positive) cells 16 days later. Third, the significant bone loss during the first 5 days was restored by day 21. These studies suggest that inhibition of RANK prevents and reverses wear debris-induced osteolysis without affecting osteogenesis. RANK.Fc has not been tested in humans to date⁴⁰.

OSTEOPROTEGERIN IN PHASE II CLINICAL TRIAL

Osteoporosis develops in OPG-knockout mice, and is corrected by OPG treatment^{29,30}. OPG treatment also corrects osteoporosis in an ovariectomized mouse model. Transgenic mice over-expressing OPG develop osteopetrosis²⁸. In the first Phase II clinical trial of OPG, Bekker et al. tested the effect of OPG in postmenopausal women. Bone collagen degradation products measured in urine showed a highly significant decrease (4-5-fold by day 4, returning to baseline by 4 weeks) following a single subcutaneous injection of 3 mg recombinant human OPG. The apparent inhibition of bone turnover rate *in vivo* suggests the potential utility of OPG in the treatment of osteoporosis. The authors noted no significant side effects, and the treatment was well tolerated by all study enrollees⁴¹. This is the only published data on OPG therapy in humans to date; clinical trials of RANK.Fc have not yet been published.

DISCUSSION

Following the description of the RANKL/RANK interaction and the discovery of OPG, there has been an explosion of research into this topic. Over 150 articles have been published on OPG, alone, since January, 2001. It is now clear that formation of the RANKL/RANK complex plays a central role in pathologic osteoclast activation seen in diseases characterized by osteolysis. This common mechanism is at work in a broad range of disorders including benign and malignant neoplasms, metabolic abnormalities, vascular disease, and prosthetic implant failure. The implications for therapies targeting this interaction are clearly far-reaching. OPG and RANK.Fc have emerged as novel therapies with great promise in the treatment of osteolytic disease. However, the available data is limited. There remains only one clinical trial studying the use of OPG, of which the initial results are encouraging. The profile of side effects and complications appears to be favorable, but more studies are necessary to ensure the safety of this novel medication. RANK.Fc has showed significant promise in animal studies, and clinical studies are anticipated.

Future directions for this research should include inquiries into endogenous OPG, RANK, and RANKL: cell sources and gene regulation, modes of sequestration, inhibition, specificity, activation, proteolytic cleavage, and clearance. For example, serum OPG levels may eventually be correlated with disease prevalence, risk of skeletal metastasis or implant failure. Elucidation of the human genetics of the RANKL/RANK/OPG system, including the specific downstream signaling molecules in different cell types, will be essential for understanding and treating a broad range of inherited and idiopathic skeletal diseases. Familial Expansile Osteolysis is now known to be an autosomal dominant disease caused by a mutation in the gene for RANK^{42, 43}. Perhaps mutations in the genes encoding RANKL or OPG are the cause of other yet-unexplained diseases. Finally, the RANKL/RANK/OPG system is an attractive target for small molecule drug design which may generate additional therapeutic options.

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