Pathophysiology of RANK ligand (RANKL) and osteoprotegerin (OPG)

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CELL BIOLOGY

Receptor activator of nuclear factor-κB ligand (RANKL) is a membrane-bound peptide of the tumor necrosis factor (TNF) ligand superfamily [14]. Rich sources of RANKL expression include T lymphocytes [12] and osteoblastic lineage cells [8]. In the presence of permissive concentrations of macrophage-colony stimulating factor (M-CSF), RANKL stimulates the differentiation, proliferation, fusion and activation of osteoclastic lineage cells, resulting in an increased number of active osteoclasts and enhanced bone resorption [14, 22]. RANKL exerts its biological effects upon activating receptor activator of nuclear factor (NF)-κB (RANK), a transmembrane receptor of the TNF receptor (TNFR) superfamily which is mainly expressed by osteoclasts and dendritic cells [11].

Osteoprotegerin (OPG) represents a soluble receptor which belongs to the TNF receptor superfamily and acts as a receptor antagonist for RANKL [21]. OPG is ubiquitously produced by a variety of tissues, cell types, and cell lines, including mesenchymal stromal cells and osteoblasts. OPG binds both the soluble and cell-bound form of RANKL and, thus, prevents their interaction with, and stimulation, of RANK [14, 21]. Consistent with this, the in vitro effects of OPG include inhibition of osteoclast differentiation, survival, fusion, and activation of osteoclasts as well as stimulation of osteoclast apoptosis, thereby reducing the pool of active osteoclasts capable of resorbing bone [21].

Over the past years, it has become clear that RANKL and OPG are essential determinants of osteoclast cell biology and bone resorption [9, 22].

ANIMAL MODELS

In vivo, treatment of mice with RANKL activates osteoclasts, promotes bone loss and causes severe hypercalcemia [14], whereas RANKL deletion results in the absence of mature osteoclasts and subsequent development of osteopetrosis [13]. Deletion of RANK in mice generates a phenotype identical to that of RANKL-deficient animals [5]. Overexpression of OPG in mice or administration of OPG to normal rodents inhibits osteoclastogenesis, osteoclast activation and bone resorption, resulting in an osteopetrotic phenotype [21]. By contrast, OPG deletion was associated with enhanced osteoclastogenesis, increased bone resorption, and massive osteoporosis [16].

In various animal models of benign and malignant bone diseases, the administration of an OPG fusion protein or soluble RANK, both of which neutralize RANKL, was able to prevent bone resorption and to reduce bone loss. These models included bone loss associated with estrogen deficiency [21], inflammatory arthritis [12], periodontal infection [23], myeloma bone disease [4, 18], humoral hypercalcemia of malignancy [17], and bone metastases of various origin [10, 15, 24].

THE RANKL/OPG SYSTEM IN HUMAN BONE DISEASES

Abnormalities of the RANKL/OPG system have been detected in various human metabolic bone diseases. Several lines of evidence implicate the OPG/RANKL system in the pathogenesis of osteoporosis following estrogen deficiency: 17β-estradiol is able to enhance OPG production in human osteoblasts through stimulating of osteoclast differentiation. Marrow stromal cells and lymphocytes from postmenopausal women display higher levels of RANKL expression than premenopausal women or postmenopausal women on estrogen replacement therapy [6]. In these women, RANKL expression is inversely correlated with serum levels of 17β-estradiol and positively with bone resorption markers. The role of serum levels of OPG and soluble RANKL and their association with meta-
bolic bone diseases, including postmenopausal osteoporosis has been recently reviewed [19].

Glucocorticoid-induced osteoporosis may result from concurrent up-regulation of RANKL expression and inhibition of OPG secretion by osteoblastic cells under glucocorticoid exposure [8]. Several studies have also documented that even short-term treatment with systemic glucocorticoids is associated with a decrease of OPG serum levels.

Myeloma bone disease is characterized by osteolytic lesions and hypercalcemia which result from enhanced osteoclastic bone resorption triggered by myeloma cells. In this process, myeloma cells employ several sophisticated mechanisms to manipulate the OPG/RANKL/RANK system [4, 7, 18, 20]. Myeloma cells concurrently augment RANKL expression and suppress OPG production by mesenchymal stromal cells through cell-to-cell interactions and secretion of dickkopf-1. The ensuing elevated RANKL-to-OPG ratio promotes osteoclast differentiation and activation in areas adjacent to myeloma cell infiltrates. In addition, myeloma cells express RANKL, and RANKL immunoreactivity is positively correlated with the number of osteolytic lesions. The expression of the proteoglycan syndecan-1 on their cell surface enables myeloma cells to internalize and degrade OPG. This mechanism leads to local inactivation of OPG, and contributes to low OPG serum levels in patients with myeloma bone disease.

**Therapeutic implications of RANKL blockade**

Based on cell biology and animal studies, it was logical to assume that RANKL blockade is feasible in human bone diseases and prevents bone loss. Two small randomized controlled trials have evaluated the short-term effects of a single dose of OPG-Fc fusion protein on biochemical bone markers. In a study in women with postmenopausal osteoporosis over 12 weeks, OPG treatment resulted in a marked and sustained suppression of bone resorption and formation markers [1]. Another study compared the effects of a single dose of OPG vs. pamidronate in patients with myeloma bone disease and in women with skeletal metastases from breast cancer over 8 weeks [3]. Urinary excretion of the bone resorption marker N-telopeptide was reduced by treatment with OPG-Fc fusion protein by 74% (breast cancer) and 47% (myeloma) which was comparable to the pamidronate effects. These short-term trials were proof-of-principle that RANKL blockade is an efficient modality to treat human metabolic bone diseases characterized by enhanced bone resorption.

More recently, denosumab, a human monoclonal antibody against RANKL has been developed. Denosumab has several advantages over OPG fusion protein. It has a higher specificity for RANKL, a longer half-life, thus limiting its administration to subcutaneous injections every 6 months, and has not been found to induce auto-antibodies. Denosumab produced a rapid, profound, and sustained decline of bone turnover markers and an increase of bone mineral density in women with postmenopausal osteoporosis [2].

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