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Bone metabolism and RANKL/RANK/OPG trail in periodontal disease

LUKASZ CZUPKALLO¹, MANSUR RAHNAMA¹, DOMINIK KIELBOWICZ¹,
MICHAL LOBACZ¹, MARYLA KOZICKA-CZUPKALLO²

¹ Chair and Department of Oral Surgery, Medical University of Lublin, Poland

² 'DentArtis' Dental Office, Lublin, Poland

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ABSTRACT

Periodontal disease is an inflammatory disease of multifactorial etiology. In order for it to appear there must come to an imbalance between the effects of pathogens and host defense mechanisms. As a result of its course the destruction of structures supporting the teeth appears (periodontium, cement, bone), and consequently leads to teeth loosening and loss. In recent years, the participation of RANKL/RANK/OPG in bone remodeling process was highlighted.

At the molecular level the bone resorption is regulated through the interaction of the ligand receptor activator of nuclear NF-kappa B (RANKL) and osteoprotegerin (OPG), which is a system of two proteins belonging to the protein tumor necrosis factor (TNF). Recent findings about the RANKL protein and OPG have shed new light on the previously unexplained phenomenon of the basis of bone resorption.

Research has shown that both protein OPG and RANKL can be detected in gingival crevicular fluid, which has become a window of opportunity in the analysis of non-invasive markers of periodontal tissues, confirming elevated levels of RANKL protein in periodontal disease, and decreased levels of OPG protein. Bone resorption is initiated by the binding of the RANKL protein to receptors RANK present on the surface of mature osteoclasts, and their precursors, which leads to the differentiation and activation of osteoclasts. OPG, being RANKL's inhibitor, has, in turn, opposite characteristics to RANKL, resulting in the reduction of osteoclastogenesis process. Despite all this, the exact mechanism of bone resorption has not yet been elucidated.

Periodontal disease is an inflammatory disease of multifactorial aetiology. Bacterial infection is crucial here. Such bacterium species as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola* or *Fusobacterium nucleatum* are among those particularly pathogenic. While gingivitis is completely reversible and does not cause any permanent changes in tissue, periodontitis is a disease that leads to destruction of gingiva, periodontium, bone and cementum, consequently leading to teeth loosening and loss. The entire process is very complex and is controlled by different proteins called 'cytokines', which are released by the pathogens and the host in response to an inflammation. The process leads, directly or indirectly, to destruction of the periodontal structures. Among these substances are the immunoglobulins, interleukins,

prostaglandins, metalloproteinases, lipopolysaccharides and many more. A healthy body maintains a balance between the etiologic factors for *periodontitis* and the host's defence mechanisms. However, there are many factors favouring the intensification and affecting the course of inflammation: poor oral hygiene, dental calculus, caries, incorrect fillings or prosthetic appliances and re-implanted teeth. Such common and well-tolerated local factors are capable of revealing harmful effects if a factor of general nature emerges, such as pregnancy, blood disease or endocrinopathy.

An imbalance between dental plaque and the body's defence can be a triggering factor for the development of *gingivitis*.

Periodontal disease is characterized by a non-homogeneous course with concomitant remissions and exacerbations. In the exacerbation phase, the tissues surrounding the teeth, including the bone tissue, are being actively damaged. This process, if not curbed at the right time, leads to loosening and subsequent loss of teeth.

* Corresponding author

e-mail: czupkallo@gmail.com

OSTEOGENIC CELLS (OSTEOBLASTS)

Osteoblasts arise from multipotent mesenchymal stem cells, and are responsible for the secretion of the organic components of the bone matrix, or collagen (type I) and proteoglycans, as well as for the synthesis of osteocalcin, osteopontin and proteinases. Their functions are also regulated by parathormone (PTH) and 1,25-dihydroxyvitamin D (1,25(OH)₂D₃) [23]. The differentiation of osteoclasts is due to modification of osteoblasts through expression of RANKL that activates the development of osteoclasts, and to secretion of osteoprotegerin (OPG) that blocks the effect of RANKL [23]. The proliferation and maturation of osteoblasts is influenced by many other growth factors (IGF1, IGF2, TGF-β1, TGF-β2, BMP, FGF, VEGF and PDGF). Pro-inflammatory cytokines, such as IL-1 or TNF, play a role in the induction of osteogenic cells and increase RANKL expression, thus causing disorders of bone metabolism.

OSTEOCLASTS

Osteoclasts are 50 to 100 μm in diameter, and have prominent Golgi apparatus and eosinophilic cytoplasm that is rich in mitochondria, lysosomes and polyribosomes. Osteoclasts also have polarity. This is due to plications within the cell membrane in the form of microvilli located on the side of the bone tissue surface (a so-called ruffled border). The ruffled border plays an important part in bone resorption that is taking place in Howship’s lacunae. Contact between osteoclasts and bone matrix is possible due to integrins αβ3 present on their surface. Their occurrence and activity can be inhibited by the following factors: oestrogens, OPG, IL-4, IL-18, TGF-β, IFN-γ, FGF-2, calcitonin and bisphosphonates [21,23]. On the contrary, IL-1, IL-6, IL-11, TNF, PGE₂, M-CSF and vitamin D3 induce osteoclast formation through RANKL production stimulated by osteoblasts and bone marrow stromal cells [23,27]. As a consequence of bone degradation, the local growth factors, i.e. TGF-β, FGF or BMP are released from the matrix to stimulate the maturation of the precursors of osteoblasts that produce osteoprotegerin (OPG), the main osteoclastogenesis inhibitor [17,18,23].

OSTEOCLASTOGENESIS

The regulation of osteoclastogenesis, and, consequently, of bone resorption, is influenced by the following factors:

- generally acting hormones,
- locally acting hormones,
- the effects of cell-cell interactions (osteoblast/bone marrow stromal cell-precursor/osteoclast) [1].

It has been found that it is osteoblasts and bone marrow stromal cells, not osteoclasts, that have receptors for the hormones responsible for the stimulation of bone resorption. These are: 1,25 (OH)₂D₃ and PTH.

Under the action of these hormones, such cells produce and release into the environment a number of factors acting in a paracrine or autocrine manner. Among these are the Macrophage Colony – Stimulating Factor (M-CSF), interleukins (IL-1, IL-6, IL-11) or prostaglandin E₂ (PGE₂).

The effects of cell-cell interactions (osteoblast/bone marrow stromal cell-precursor/osteoclast), in which relevant receptors and ligands are bound to cell membranes, are of great importance for the osteoclastogenesis and the activation of mature multinucleated osteoclasts. This system consists of three elements: a RANK receptor, osteoprotegerin and a ligand for the RANK receptor (RANKL) [1].

RANKL/RANK/OPG PATHWAY

Proper osteoclast maturation and function are dependent on a pathway comprising osteoprotegerin (OPG), the Receptor Activator of Nuclear Factor – κ B (RANK) and its RANK ligand (RANKL – Receptor Activator of Nuclear Factor – κ B ligand) [4,10,25]. A fully active osteoclast is formed due to the activation of a cascade mechanism inside a maturing bone resorption cell, resulting from the binding of the Receptor Activator for Nuclear Factor κ B ligand (RANKL) with the Receptor Activator of Nuclear Factor κ B (RANK) on the surface of osteoclast precursor cells. This process involves osteoprotegerin (OPG), which is a protein from the tumour necrosis factor receptor family [8,24]. The expression levels of RANKL (increase of resorption) and OPG (increase of bone formation) proteins can be influenced by a number of substances and cytokines (Table 1) [25].

Table 1. The impact of different factors on the level of expression of OPG and RANKL (table description: 0 – no change; ↓ – inhibit; ↑ – stimulate; ? – no scientific reports) [1,19]

Cytokine	Increase of resorption	Increase of bone formation
BMP2-7	0	↑↓
TGF-β	↓	↑
IGF-1, IGF-2	0	↑
FGF	0	↑↓
PTH	?	?
PDGF	↑	↑
TNFα	↑	↑
IL-1	↑	↑↓
IL-3	↑	?
IL-6	↑	?
PGE ₂	↑	↑↓
INFγ	↓	↑
17 β-estradiol	↓	↑
1,25(OH) ₂ vit.D3	↑	↑
Calcium	↑	↑
Glucocorticoids	↑	↓
Immunosuppressants	↑	↓

OSTEOPROTEGERIN

Osteoprotegerin (OPG) is a protein that was first described in 1997. It was identified as a secreted molecule that inhibits the differentiation and activity of osteoclasts, i.e. as an osteoclastogenesis inhibitory factor [1,4,25]. OPG is a member of the tumour necrosis factor receptor (TNFR) superfamily and is synthesized predominantly by osteoblasts and many organs (lungs, liver, intestines, kidneys, stomach,

skin, bones), as well as by periodontal tissues, gingival epithelium and gingival fibroblasts [5,23]. Like RANKL, OPG is modulated by the inflammatory cytokines present in *periodontitis* [2].

Osteoprotegerin is a dummy receptor that competitively binds to RANKL/OPGL [28]. This prevents osteoblasts and bone marrow stromal cells from contacting both the precursors and the mature multinucleated osteoclasts having a RANK receptor, and results in inhibition of osteoclastogenesis (Figure 1).

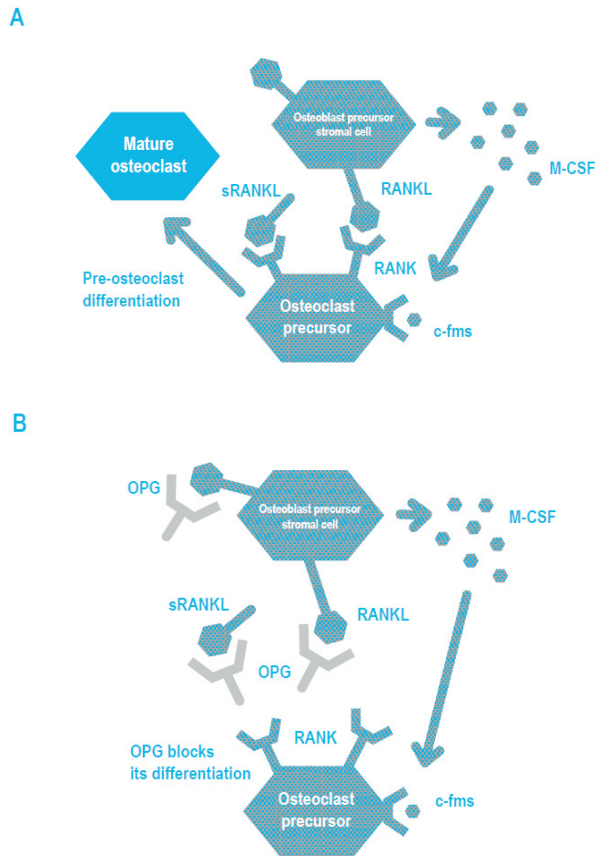


Figure 1. Participation of proteins of the RANK/RANKL/OPG system and other osteolytic factors in the regulation of osteoclastogenesis. A-activation of differentiation of the osteoclast precursor by RANKL and M-CSF; B-inhibition of the differentiation and maturation of osteoclasts by osteoprotegerin [1,2]

OPG expression is promoted by many factors, such as interleukins (IL-1 α , IL-6, IL-11, IL-13, IL-18), growth factors (PDGF, TGF- β 1), 17 β -estradiol, OSM (oncostatin M) or mechanical loads exerted on bone [19]. In contrast, OPG inhibitors are: parathormone (PTH), PGE₂, immunosuppressive drugs, glucocorticoids, vitamin D₃ and its derivatives [1,2,4,19,20,22].

RANK LIGAND (RANKL)

RANKL (Receptor Activator for Nuclear Factor κ B Ligand) belongs to the protein superfamily of tumour necrosis factors (TNF) and is a product of mature osteoblasts, along with their precursors, fibroblasts, chondrocytes, endothelium, bone marrow stromal cells, malignant cells or active T lymphocytes. It is a factor that activates formation of mature osteoclasts: their differentiation, fusion,

function and survival. RANKL acts via the RANK receptor (Receptor Activator for Nuclear Factor κ B) present on the cell surfaces [1]. RANKL activates osteoclastogenesis by blocking apoptosis of osteoclasts, while binding to RANK which is present on the surface of resorption cells, or osteoclasts [6,23] (Figure 2).

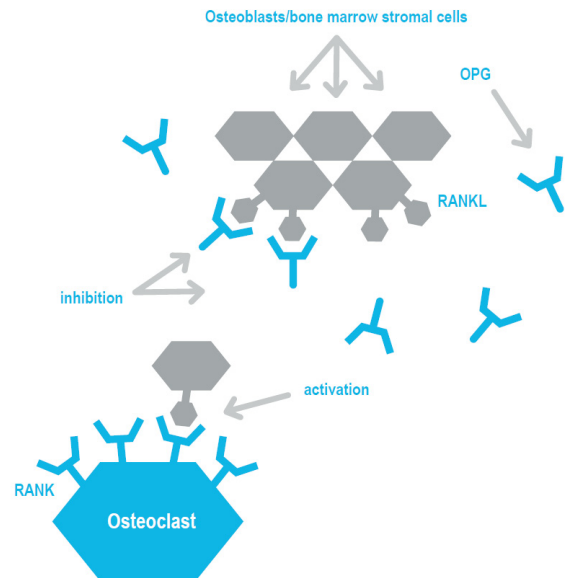


Figure 2. RANKL/RANK/OPG interaction. RANKL-RANK binding on pre-osteoclasts is essential for their maturation and subsequent activity. With the help of M-CSF, RANKL activates osteoclastogenesis by blocking apoptosis of osteoclasts while binding to the RANK present on the surface of resorption cells, or osteoclasts [1,2]

RANKL gene expression is blocked by 17 β -estradiol, and by IL-13 or TGF- β 1 as well [19,23]. Moreover, 1,25(OH)₂D₃, IL-1 β , IL-6, IL-7, IL-11, IL-17, TNF, PTH, OSM, IFN- γ , PGE₂, vitamin D₃, glucocorticosteroids and dexamethasone promote an increase in gene expression for RANKL in the presence of M-CSF [1,19,23,25].

RECEPTOR ACTIVATOR OF NUCLEAR FACTOR - K B (RANK)

RANK is a type I glycoprotein from the TNF receptor superfamily that is the receptor RANKL, or the most important cytokine that is responsible for the development and maturation of osteoclasts. It is a transmembrane protein that can be found in mature osteoclasts and their precursors, fibroblasts, T and B lymphocytes, chondrocytes, endothelial cells, dendritic cells, and in malignant cells as well [1,2,11,13,19,23,28]. Increased RANK expression is due to the activity of RANKL, IL-1, MIP-1 α , VIP, oncostatin M (OSM), vitamin D₃, TGF- β and IFN- γ , whereas the reduction of the number of RANK molecules on the surface of osteoclasts may be induced by IL-4 [1,2,4,23,26].

RANKL AND OPG EXPRESSION IN PERIODONTAL DISEASE

Numerous studies showed that IL-1, IL-6 and TNF- α , as well as other inflammatory cytokines, are significant in

periodontal disease due to the fact that they control RANKL and OPG expression [3,25]. OPG and RANKL are regarded as the main bone metabolism regulators and are also considered to be crucial for the mechanism of periodontal destruction in *periodontitis* [3].

Inflammatory cell infiltration in chronic periodontal disease leads to bone atrophy in *periodontitis*. This situation is reflected in increased RANKL and decreased OPG in the inflammatory granulation tissue adjacent to the atrophied bone. [3,25]. According to Nagasawa *et al.*, RANKL expression is higher in deeper periodontal pockets, compared to the physiological gingival pockets [15,16]. Liu *et al.* also confirmed that the RANKL level was higher in people with the advanced stage of periodontal disease, compared to those with moderate *periodontitis* or those who are healthy [4,12,15].

RANKL mRNA expression was observed in inflammatory cells, mainly in lymphocytes and macrophages. Furthermore, the proliferating epithelium in the region of pro-inflammatory cells also exhibited increased expression of RANKL mRNA [2,15].

Using immunohistochemical methods, Crotti *et al.* compared RANKL and OPG expression in the inflammatory granulation tissue adjacent to the places of bone atrophy in periodontal patients with the parodontal tissues, and in patients without any symptoms of periodontal disease [2,15]. Herein, the level of RANKL proteins was significantly higher in tissues with clinical parodontopathy, whereas the OPG level turned out to be clearly lowered [2,15,25].

Mogi *et al.* measured RANKL and osteoprotegerin concentrations in Gingival Crevicular Fluid (GCF) in patients with *periodontitis* and in healthy ones. Increased RANKL and decreased OPG were obtained for the group with diagnosed *periodontitis*. Furthermore, the ratio of the concentration of RANKL to that of OPG in Gingival Crevicular Fluid was significantly higher in that group, compared to the ratio found for the group of healthy people [14,15].

The RANKL/OPG ratio can be seen as the main determinant of bone metabolism. In many situations, bone absorption is stimulated by both the increased RANKL level and the decreased OPG level which act as pro-resorptive signals [9].

The mechanisms behind the bone resorption process in periodontal disease is still being investigated. Progress in medicine is extremely fast, and medical discoveries made ten years ago often become anachronisms. Still, a willingness is triggered amongst researchers to discover what has not been fully investigated yet. Amongst such targets are (for example) the regulation effect and mechanisms in other physiological and pathological situations of the organism.

Currently, sRANKL and OPG as inflammatory process markers, seem to have as yet limited practical applications due to the multitude of tissues that are sources of the proteins in question. However, the role of RANKL inhibitors as therapeutic agents for treatment of conditions involving bone mass loss, as for example postmenopausal osteoporosis, myeloma or breast carcinoma, is promising [9,10].

DECLARATION OF INTEREST STATEMENT

No competing financial interests exist amongst the researchers contributing to this article.

ABBREVIATIONS THAT WERE USED IN THE TEXT:

BMP – Bone Morphogenetic Protein; GCF – Gingival Crevicular Fluid; FGF – Fibroblast Growth Factor; IFN- γ – Interferon gamma; IL – Interleukin; IGF – Insulin-Like Growth Factor; M-CSF – Macrophage Colony-Stimulating Factor; NF- κ B – Nuclear Factor kappa B, Nuclear Factor kappa-light-chain-enhancer of activated B cells ; OPG – Osteoprotegerin; OPGL – Osteoprotegerin Ligand; OSM – Oncostatin M; PDGF – Platelet-Derived Growth Factor; PGE₂ – Prostaglandin E₂; PTH – Parathyroid hormone; RANK – Receptor Activator of Nuclear Factor – κ B; RANKL – Receptor Activator of Nuclear Factor – κ B ligand; sRANKL – soluble RANKL; TGF- β – Transforming growth factor β ; TNF – Tumor Necrosis Factor; TNFR – Tumor Necrosis Factor Receptor; VEGF – Vascular Endothelial Growth Factor

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