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Osteoclastogenesis regulatory factors (RANK, RANKL and OPG) in osteolytic jaw lesions

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Abstract

Receptor activator of nuclear factor κB (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) are members of the superfamily of ligands and receptors of tumor necrosis factor relationed with bone metabolism. Osteoclastogenesis is regulated by these three proteins. The aim of this review was to assess the immunohistochemical expression of these proteins in osteolytic jaws lesions. Studies were identified by searching MEDLINE/Pubmed. The results link higher imunoexpression for RANKL compared to OPG in chronic periodontitis and in aggressive odontogenic tumors, and in less aggressive odontogenic tumors a tendency for higher expression for OPG. Taken into account this, OPG could be a good candidate for the treatment of oral lesions can often be widely destructive.

Keywords: Osteoclastogenesis; RANKL; RANK; OPG

Fatores reguladores da osteoclastogênese (RANK, RANKL e OPG) em lesões osteolíticas da maxila

Resumo

Receptor activador do factor nuclear κB (RANK), ligante de RANK (RANKL) e osteoprotegerina (OPG), são membros da superfamília de ligantes e receptores do fator de necrose tumoral relacionados com o metabolismo ósseo. A osteoclastogênese é regulada por estas três proteínas. O objetivo desta revisão foi avaliar a expressão imuno-histoquímica destas proteínas em lesões osteolíticas dos maxilares. Os estudos foram identificados através de pesquisa MEDLINE/PubMed. Os resultados revelaram maior imunoexpressão para RANKL em relação a OPG em periodontites crônicas e em tumores odontogênicos agressivos, já em tumores menos agressivos houve uma tendência de maior expressão para OPG. Levando em conta estes achados, a OPG poderia ser um bom candidato para o tratamento de lesões maxilares que podem muitas vezes ser amplamente destrutivas.

Palavras-chave: Osteoclastogênese; RANKL; RANK; OPG

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Introduction

Bone is a dynamic tissue that is continually remodeled in intrinsic mechanism that integrates external chemical, hormonal and biomechanical stimuli [1]. At the cellular level, bone remodeling allows a result of repeated cycles of bone resorption by osteoclasts which are derived from the macrophagic lineage, followed by bone formation driven by osteoblasts [1,2]. Most of the bone diseases are caused by a disturbance in the number and activity of osteoclastic cells, resulting in improper bone resorption which exceeds the compensatory capacity of osteoblasts [1-4]. Increased osteoclast activity is seen in many osteopenic disorders such as postmenopausal osteoporosis, Paget's disease, bone metastases and rheumatoid arthritis [5].

Cytokines and growth factors for the differentiation and maturation of osteoclasts in humans include interleukin-1 (IL-1), IL-3, IL-6, IL-11, tumor necrosis factor (TNF), colony-stimulating factor granulocyte-macrophage (CSF-GM) and macrophage colony-stimulating factor (M-CSF). Besides stimulating cell proliferation, growth factors may have an effect on locomotion, contractility, cell differentiation and angiogenesis. These growth factors act on osteoclastogenesis by stimulating stem cells or paracrine system, essential for bone metabolism, where osteoblasts and progenitor cells play a central role as mediators through receptor activator nuclear κappa B (RANK), receptor activator nuclear κappaB- ligand (RANKL) and osteoprotegerin (OPG) [6].

RANK, RANKL and OPG are key regulators in osteoclast biology and bone metabolism [7,8]. RANKL interacts with its receptor RANK located on osteoclast precursors and dendritic cells, and activates c-Jun, NFkβ pathways that are related to the process of differentiation, proliferation and activation of osteoclasts [1]. The effects of RANKL are blocked by soluble decoy receptors such as OPG that competes with RANK for binding to RANKL [1,2,7]. In vitro and in vivo studies have shown that RANK/RANKL/OPG are essential for the life of osteoclasts and, as mediators of bone diseases, are important molecular targets for diagnosis and therapeutic intervention [1,6].

The aim of this review is to look for immunohistochemical expression of these three proteins in osteolytic jaw lesions, contributing to knowledge of these proteins in these lesions and their possible clinic applications as treatment option.

RANK

RANK is a type 1 transmembrane receptor (cell surface) of the TNF family that is present in osteoclast precursor cells, dendritic cells, fibroblasts and T cells. RANK human is a peptide of 616 amino acids. The activation of RANK by RANKL is followed by its interaction with family members of the TNF receptor associated (TRAF), activation of NF-κB and c-Fos, which are related to the process of osteoclast maturation [3]. When activated, RANK promotes the maturation of osteoclasts by increasing the expression of specific genes [3,8-10]. The RANK signaling cascade is complex and poorly understood and its role in osteoclastogenesis and proliferation of tumor cells is being investigated in an attempt to find a target for future anti-tumor therapies [11].

RANKL

RANKL is a peptide of 317 amino acids of the TNF family, expressed differently as a cytokine of cell membrane or as a soluble factor released by several cell types such as T lymphocytes and osteoblasts [8,11,12]. While the cell surface form is more common and expressed by various cell types, the secreted form is restricted to activated T cells and squamous cell carcinoma cell lines [1,12,13].

RANKL, also called osteoprotegerin ligand (OPGL), osteoclast differentiation factor (ODF) or TNF-related activation cytokine inducer (TRANCE), acts as ligand of OPG and played immuno-modulatory activity since RANKL-deficient mice develop lymph node agenesis and thymic hypoplasia. The RANKL is considered a stimulator of dendritic cells, acting as a survival factor for dendritic cells and modulating the activation of mature T cells. These activities are associated with activation of NF-κB after the binding of RANKL to its membrane receptor RANK [1,14].

The biological effects of RANKL are produced when it binds to RANK on the cell surface of pre-osteoclasts resulting in fusion, differentiation, survival and activation of osteoclasts [9,10,12]. In the presence of permissive concentrations of M-CSF, RANKL stimulates the differentiation, proliferation, fusion and activation of osteoclastic lineage cells, resulting in an increase in the number of active osteoclasts and bone resorption [3,12].

The expression of M-CSF by osteoblasts is required for progenitor cells to differentiate into osteoclasts. The cell alone is unable to complete this process. M-CSF bound to its receptor (rM-CSF or c-Fms) functions as a primary signal for the development of osteoclasts. The complete differentiation of osteoclasts requires the expression of RANKL by osteoblasts and RANK by osteoclast precursors [3,11].

In vivo RANKL experiments promote activation of osteoclasts, causing bone loss and severe hypercalcemia, while the deletion of RANKL results in the absence of mature osteoclasts and subsequent development of osteopetrosis. Deletion of RANK in mice generates a phenotype identical to that of RANKL-deficient animals. These findings suggest that RANKL is a pro-resorptive factor [8,14].

The cellular response of RANKL depends on the presence inhibitory receptor, OPG, as well as the level of expression of its receptor RANK, which is primarily expressed in cell line macrophages/monocytes, including osteoclast precursors, T and B cells, dendritic cells and fibroblasts (9). RANKL activates its specific receptor RANK signaling

a cascade of signals that involve the stimulation of c-jun, NF-kB, and serine/threonine kinase (PKB/Akt) that are related to proliferation, differentiation, and apoptosis [1].

The effects of RANKL are opposite to osteoprotegerin (OPG). RANKL and OPG are regulated by various hormones (glucocorticoids, vitamin D, estrogen), cytokines (tumor necrosis factor alpha, interleukin 1, 4, 6, 11 and 17), and several mesenchymal transcriptional factors [1].

OPG

The biggest inhibitor of osteoclastogenesis was identified simultaneously by the group Tsuda and Amgen company in 1997-1998 [1,11,14]. They named, respectively, this new negative regulator of osteoclast differentiation, as osteoclastogenesis inhibitory factor (OCIF) or osteoprotegerin (OPG). OPG is a peptide of 380 amino acids belonging to the TNF receptor family, and in contrast to all other TNF receptors lack cytoplasmic and membrane domains, and are secreted as soluble proteins [3,12].

OPG is a soluble receptor that acts as an antagonist of RANKL [3]. It is produced by numerous cell types including immune cells, osteoblast and endothelial cells. It is considered an inhibitory receptor for RANKL, because it blocks the interaction RANK/RANKL, inhibiting the terminal stage of osteoclast differentiation and thereby resulting in decreased bone resorption [1,14].

In vitro studies demonstrate that the effects of OPG include inhibition of differentiation, survival and osteoclast fusion, as well as stimulation of apoptosis of osteoclasts, thereby reducing the ability of bone resorption [3,8,12,15]. Super-expression of OPG or administration of OPG in mice inhibits osteoclastogenesis, activation of osteoclasts and bone resorption, resulting in a phenotype osteopetrotic. Moreover, the deletion of OPG was associated with marked osteoclastogenesis, increased bone resorption and massive osteoporosis [3,8,15].

The role of OPG was demonstrated through experiments with transgenic and knock-out mice. Osteoprotegerin knock-out mice develop a large decrease in density and bone volume, and suffer a osteoporosis associated with a high incidence of fractures and deformities in the vertebrae. This induced osteoporosis was completely reversed by intravenous injection of recombinant OPG. This demonstrates, that the presence of OPG, is essential for maintaining bone mass in physiological functioning as a powerful agent for osteoprotection [14,15].

The osteoprotective role of OPG has also been supported by partial deletion of this protein in patients with juvenile Paget's disease, an autosomal recessive disease in which affected individuals have an increased bone turnover, osteopenia, and fractures [11]. An increased activity of RANKL, associated with a decrease of regulatory activity of OPG, has been implicated in several diseases such as osteoporosis, rheumatoid arthritis and periodontal disease [1,9,10,16,17].

RANK/RANKL/OPG in jaw lesions

These markers have been used in several studies of osteolytic lesions and have been highlighted as molecules responsible for bone turnover. The RANKL/OPG ratio may be used as a biological marker of prognosis in bone injuries such as osteoporosis, ankylosing spondylitis, rheumatoid arthritis, benign bone tumors and prosthetic osteolysis associated with loss and bone fractures. This index may also be important to assess new drugs for bone disease and as therapy for bone pathologies [14]. RANKL and OPG are also important regulators of vascular biology, calcification and formation of mammary glands during pregnancy, indicating its crucial role in the management of extra-skeletal calcium [1].

Recent studies have confirmed that RANKL and OPG can be detected in gingival crevicular fluid and indicate that the level of RANKL is increased while that of OPG is decreased in periodontitis or during orthodontic movement. An increase in the RANKL/OPG ratio may indicate bone destruction. It is hypothesized, for example, by increasing this ratio in gingival crevicular fluid from patients with periodontitis [18,19]. Lu et al. [20] showed that RANKL-positive cells were significantly distributed in the inflammatory connective tissue zone of disease gingiva, compared with those of samples from non-diseased persons. However, few OPG-positive cells were found in both tissues. Dereka et al. [21] noted RANKL as well as OPG expression were reduced in chronic periodontitis, but the RANKL/OPG ratio showed to be slightly elevated in chronic periodontitis. Then, further investigation is needed to identify the specific role of RANKL and OPG protein in chronic periodontitis, since contradictory results has been obtained.

Odontogenic cysts and tumors are one of the most common osseous-destructive lesions of the jaws. Numerous biomarkers have been used for therapeutic interventions research. In several animal models of benign and malignant bone diseases, the administration of the OPG protein or soluble RANK was able to neutralize the RANKL and thus preventing bone resorption and reduce bone loss [3]. Andrade et al. [9] evaluated the expression of these markers in the epithelium and stroma cells of odontogenic tumors and found no differences in epithelial immunostaining between the lesions. In the stroma, they obtained different results with a group of lesions showing values of OPG higher compared to RANKL in lesions less aggressive (calcifying cystic odontogenic tumor and adenomatoid odontogenic tumor), and in others group, RANKL levels higher than the OPG (calcifying epithelial odontogenic tumor, ameloblastic fibroma and odontogenic myxoma), indicating that the presence of immunopositivity for these markers could be related to the process of tumor invasion and bone resorption. Silva et al. [16] found in most of their samples of ameloblastomas higher levels of RANKL than OPG. In contrast, Kumamoto and Ooya [22] found higher levels of OPG than RANKL in tumor cells of ameloblastomas.

Nonaka et al. [23] analyzed imunoexpression of RANKL and OPG in syndrome (SOK) and non-syndrome odontogenic keratocysts (NSOK) and found no differences in the epithelial lining and fibrous capsule. The most cases present a similar (RANKL=OPG) or a RANKL<OPG expression in the linning epithelium of the NSOK and SOK, respectively. In the fibrous capsule, the most cases presented a similar imunoexpression in both cases.

Silva et al. [16] analyzed the immunohistochemical expression of RANKL and OPG in epithelial and mesenchymal cells in dentigerous cyst (DC). In the epithelium they found a similar distribution between cases, where 42.9% of immunopositive cells had RANKL=OPG, other 42.9% OPG<RANKL. In the stroma they verified in 100% of cases OPG>RANKL. So, they concluded that these differences between the immunostaining of these proteins in the capsule of DC, mainly in fibroblasts, endothelial cells, bone cells and osteoclast precursors identifies these cells as major source of these molecules. Moraes et al. [6] found a similar imunoexpression (RANKL=OPG) in lining epithelium of radicular and dentigerous cysts. In contrast, in the fibrous capsule they found a higher imunoexpression for RANKL than OPG in dentigerous cysts compared with radicular cysts. This finding could be explained by increased vascular permeability characterized by the presence of hemorrhagic areas in the capsule of DC. Min et al. [24] showed that vascular endothelial growth factor (VEGF) up regulates expression of RANK and increases angiogenic responses of endothelial cells to RANKL.

Menezes et al. [10] found positive neutrophils, macrophages, endothelial cells, epithelial cells and lymphocytes in radicular cyst for RANKL and OPG. Based on the expression patterns of RANKL/OPG in different physiological and pathological conditions, it is suggested that sites of active bone resorption have different pattern of expression of RANKL/OPG when compared with sites where bone resorption is absent or minimal. In inflammatory conditions, there is an imbalance in the levels RANKL/OPG what becomes excessive osteoclastic activity and pathologic bone resorption [25]. Vernal et al. [17] and Menezes et al. [25] believe that RANKL plays a key role in pathological events associated with periapical bone destruction and methods of controlling the activity of RANKL may be useful for the treatment of these cysts.

Additionally, RANKL can play important role in inducing giant cell accumulation and osteolysis in central giant cells lesion (CGCL). Itonaga et al. [26] analyzed the phenotype of multinucleated giant cells, as well as the expression of RANKL and OPG in CGLC. They noted an immunopositivity for RANKL in stromal mononuclear cells. In addition, they observed OPG markedly inhibited the formation of multinucleated giant cells and bone resorption. With these findings the authors suggested multinucleated giant cells are osteoclast-like from monocyte-macrophage precursors, which differentiate into osteoclasts under the influence of the lesion mononuclear cells which express RANKL. Thus, any therapeutic agent that inhibits the interaction between these molecules, such as OPG, could be used in the treatment of central giant cell lesions. In consonance with this, Tobón-Arroyave et al. [27] evaluated the expression of RANK, calcitonin receptor (CTR) and glucocorticoid receptor (GR) in LCCG aggressive and non-aggressive. They observed moderate to intense immunopositive RANK in multinucleated giant and ovoid mononuclear cells, and absent in spindle stromal cells. In this study, the authors suggest that the mechanism by which multinucleated giant cells and mononuclear cells induce bone resorption is similar to that found in normal bone tissue osteoclastogenesis by binding RANK/RANKL.

Following this reasoning, the mechanism of oral squamous cell carcinoma (OSCC) invading jaw bone has been few investigated. Chuang et al. [28] reported the expression of RANK, RANKL and OPG in OSCC with and without bone invasion and compared with samples of normal oral mucosa. They found strong cytoplasmic immunostaining for RANKL in the cancer cells of both groups and in osteoclasts for all cases with bone invasion. The RANK expression was similar between OSCC with and without bone invasion and absent in all normal tissues. However, for OPG they observed weak to negative immunoreactivity in OSCC, but absent in all normal tissue. It has been suggest that up-regulation of cytokines, such as tumor necrosis factor (TNF), in OSCC [29] could up-regulate the expression of RANK, because RANK is a TNF receptor.

Final Considerations

Kinetics of RANKL, RANK and OPG expressions in oral lesions varies in immunohistochemical researches. Studies have proved RANK/RANKL/OPG system to be an important signal pathway regulating osteoclastogenesis, and inactivation of RANKL, which blocks the RANK/RANKL pathways, is critical in anti-bone resorption. However, the immunohistochemical evaluation in oral lesions not always corroborates this finding and in fact the system RANK/RANKL/OPG acts directly in the process of bone resorption present in these lesions is still unclear. However, with the studies presented here we observe that the presence of cells expressing these proteins in a way that suggests their role in osteoclastogenesis, like in chronic periodontitis, odontogenic cysts and tumors, CGCL and OSCC. Particularly, in odontogenic tumors more aggressive there was higher expression of RANKL and less aggressive there was higher expression of OPG. Performing a large animal study model, recombinant OPG could be incorporated into degradable carriers such as microspheres, micelle, and delivered into experimental induced periradicular lesions in the animals, for example, by controlled release, where it can relieve bone destruction by inhibiting osteoclastogenesis and osteoclast function. Since OPG can block the RANK/RANKL pathways, it has been demonstrated could be a good candidate against bone destruction and could be widely used in the treatment of osteolytic oral lesions can often be widely destructive.

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