IJAE
Vol. 119, n. 1: 44-48, 2014
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Review in Histology and Cell Biology

Targeting RANKL in the management of bone loss in patient with breast cancer

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Submitted July 18, 2013; accepted October 18, 2017

Abstract

The receptor activator of nuclear factor-κB ligand (RANKL), its signaling receptor RANK, and its natural decoy receptor OPG are members of the tumour necrosis factor (TNF) and TNF receptor superfamily and are best known for their essential role in controlling osteoclastogenesis. RANKL in bone has also been shown to serve as a chemoattractant for cancer cells, thus explaining the tropism of certain cancers such as breast and prostate cancer to preferentially metastasize to bone. Here, we will discuss the important role of RANKL and its possible role in the management of bone loss in patients with breast cancer.

Key words

Receptor activator of nuclear factor-κB ligand, osteoclast differentiation factor, osteoprotegerin ligand (OPG-l), breast cancer, TNF, tumour necrosis factor, TNF receptor, metastasis.

Introduction

The receptor activator of nuclear factor-κB ligand (RANKL) is a member of the tumour necrosis factor (TNF) superfamily of cytokines. The protein was initially identified as a cytokine with an ability to stimulate T cell and dendritic cell function and was termed TRANCE, for TNF-related activation-induced cytokine, or alternatively as RANKL. It was cloned independently by two groups and for its ability to stimulate osteoclast differentiation, activity, and survival it was given the names osteoclast differentiation factor (ODF), and osteoprotegerin ligand (OPGL). TNF family members mediate a panoply of biological phenomena, modulating essential aspects of inflammation, organogenesis, host defence, autoimmunity and apoptosis. In general, TNF family cytokines demonstrate pleiotropic capabilities in coordinating the development and function of many disparate tissues and cell lineages. RANKL, in particular, assumes prominent roles in bone, the immune system, and mammary epithelium. The expression of both RANKL and RANK has been observed in primary breast cancers in humans and breast cancer cell lines, and we and others have proposed that the RANKL/RANK system can regulate bone metastases of epithelial tumours (Nicolin et al., 2008). Breast cancer frequently metastasizes to the skeleton. It is estimated that 85% of individuals with advanced disease harbour bone metastases. While

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http://www.fupress.com/ijae
DOI: 10.13128/IJAE-14639

ductal carcinoma in situ is 98% curable if detected early, bone metastases are basically incurable (Jemal et al., 2007). Metastatic cancer cells tend to colonize the heavily vascularized areas of the skeleton, such as the red marrow of the long bones, sternum, pelvis, ribs and vertebrae, where they disrupt not only bone physiology but also haematopoiesis and the immune system (Mundy et al., 1999).

Discovery of osteoprotegerin, RANKL, and RANK

The receptor activator of nuclear factor-κB ligand, also called TRANCE, ODF and OPGL, is a type II transmembrane protein belonging to the TNF superfamily, whose gene was cloned fifteen years ago by four different groups contemporaneously. OPG was discovered independently by two groups, although the manner in which these groups identified it differed markedly. The Amgen group in the U.S.A. initially described a molecule that they had found within a project directed at characterizing cDNAs in rat intestine (Simonet et al., 1997). Mice over-expressing one particular cDNA developed marked osteopetrosis because they did not have any osteoclasts in their bones (Yasuda et al., 1998). The protein encoded by the gene was named osteoprotegerin (the bone protector), because it appeared to protect the skeleton from excessive bone resorption by limiting osteoclastic bone resorption (Lacey et al., 1998, Yasuda et al., 1998a; Yasuda et al., 1998b). Independently, the Snow Brand Milk Group in Japan reported the identification of the identical molecule, but the route by which they got there was different. In 1981, Rodan et al. (1981) have proposed a novel hypothesis wherein the osteoblast played a central role in mediating the hormonal control of osteoclastogenesis and bone resorption. Soon after, OPGL/ODF was identified as a ligand for OPG and the cellular receptor was identified as being identical to the previously identified RANK, which had been discovered by Anderson et al. (1997) at Immunex (U.S.A.) while they were sequencing cDNAs from a human bone marrow derived myeloid dendritic cell cDNA library. They found that RANK had partial homology to a portion of the extracellular domain of human CD40, a member of the TNF receptor superfamily, and that it was involved in the activation of T cells in the immune system. They then isolated RANKL by direct expression screening and found, like Wong et al. (1997), that it increased dendritic cell stimulated naïve T cell proliferation and the survival of RANK-expressing T cells.

RANKL structure

Human RANKL is a 317-amino acid peptide that has approximately 30% homology to the TNF-related apoptosis-inducing ligand and to CD40, and approximately 20% homology to Fas ligand (Lacey et al., 1998). It has now been shown to exist in two forms: a 40 to 45 kDa, membrane-bound, cellular form and a 31 kDa soluble form derived by cleavage of the full- length form at position 140 or 145. RANKL mRNA is expressed at highest levels in bone and bone marrow, as well as in lymphoid tissues (lymph nodes, thymus, spleen, fetal liver and Peyer's patches). Its major role in bone is the stimulation of osteoclast differentiation and activity and the inhibition of osteoclast apoptosis (Anderson et al, 1997). Indeed, in the presence of low levels of macrophage-colony stimulating factor (M-CSF), RANKL appears to be

both necessary and sufficient for the complete differentiation of osteoclast precursor cells into mature osteoclasts .

RANKL and breast cancer

Accumulating evidence has shown that RANKL is not only important for normal bone development, but is implicate in a spectrum of skeletal diseases characterized by excessive osteoclastic activity, including osteoporosis, rheumatoid arthritis and bone metastases. The typical tumour-induced bone lesion is focal osteolysis, although some tumours such as breast cancer metastases exhibit osteogenic bone lesions. The RANKL/RANK pathway may direct breast cancer cells to preferentially migrate into bone, being a crucial requirement and initial step for skeletal metastasis. Epithelial cells from normal mammary glands express RANK, and RANKL-RANK signalling is required for the development of lactating mammary glands during pregnancy. Both RANKL- and RANK-deficient mice lack lactating mammary glands and cannot feed their off-spring. Based on high constitutive RANK expression in breast cancer specimens and cell lines, recent data indicate that the RANK expression status of cancer cells determines whether tumours predominantly migrate into bone, where the corresponding ligand RANKL is abundantly expressed. The correlation of high RANK expression with osteotropism in murine models was demonstrated across diverse tumour cell types, including breast cancer and melanoma. The osteolysis induced by these tumours affects the quality of life of the patients by causing pathological fractures and severe bone pain and often exacerbates the prognosis of the patients. Concomitantly, production of a soluble decoy receptor for RANKL, i.e. OPG, may be downregulated, thus eliminating one mean by which the ensuing osteolysis could be repressed. The imbalance in the RANKL:OPG ratio that results in RANKL upregulated activity leads to increased osteoclastogenesis and consequently bone loss (Narducci et al., 2011). The resulting vicious cycle is potentially inhibited by OPG, which may lead to the development of compounds that inhibit the RANK/RANK-ligand pathway as therapeutic agents.

Clinical applications

As the mechanisms involved in bone metastases are further elucidated and the pathways involving many of the previously noted molecules are more clearly understood, the clinical applications of this knowledge can have a significant impact. As previously mentioned, treatment for bone metastases, including surgery, radiation and bone-modifying agents such as bisphosphonates and denosumab, have been palliative in nature, attempting to slow disease progression, palliate symptoms and increase survival. Some of the molecules, such as RANKL, involved in metastasis-induced bone resorption are disease-specific, but many appear to be part of a final common pathway that directly increases the number and activity of osteoclasts leading to bone destruction (Nicolin et al., 2008) . The development of targeted agents for specific molecules in this final common pathway, as evidenced by denosumab and the RANKL pathway, has the potential to not only palliate but also possibly prevent the development of bone metastases and prolong patient survival (Casas et al., 2013).

Treatment and prevention of bone metastasis

Currently, the most promising results of targeted therapy for osseous lesions come from trials involving drugs that modulate the derangement of the balance in the RANK/RANK-ligand/OPG pathway that is a major driving force in the development of malignant bone lesions. Following the discovery of OPG, it was thought that increasing OPG levels would be an effective way to inhibit the bone resorbing effects of RANKL. Administration of an Fc-OPG construct seems promising as a potential therapy in animal models of bone metastasis. Accordingly, a genetically engineered recombinant OPG-Fc construct (AMGN-0007) was developed as a potential therapeutic agent for patients with bone metastases. However, there were some concerns that prevented further development of this drug in clinical trials. This agent had a short half-life, which raised some concerns on dose scheduling for clinical use. Furthermore, OPG is not specific to RANKL, as it can also block TRAIL (TNF related apoptosis inducing ligand) which is another ligand belonging to TNF family (Emery et al., 1998). TRAIL is considered a very important component in natural immunity against cancer and is the principal mediator of tumour cell death (Nicolin et al., 2010) Thus binding of pharmacological doses of OPG to TRAIL may protect breast cancer cells from undergoing TRAIL-induced apoptosis (Neville et al., 2004, 2010). This may bear a potential risk of tumour growth with the long-term use of this drug. Therefore, as an alternative approach, an antibody specific to RANKL was developed which simulates the beneficial effects of OPG on bone health while avoiding any potential reaction with TRAIL (Nicolin et al., 2010). Some epidemiologic studies suggest an anti-neoplastic role, due to the RANKL modulation, of flavonoids against breast, colon, prostate cancer. Flavonoids are phenol compounds present in the pigments of fruit, vegetables, green tea and red wine. Flavonoids have two aromatic rings (ring A and ring B) linked by a bridge composed of three carbon atoms. Depending on their state of oxidation and functional groupings, flavonoids are classified in flavons, flavonons, flavonols, isoflavons, catechines, anthocyanidines and calchones. The main flavonoids are quercetin (extracted from onion and grapes), genistein (from soya beens), apigenin (from parsley and celery), luteolin (broccoli), epicatechin, proanthocyanids, kaempferol (broccoli, grapefruit), catechins (green tea), resveratrol (grapes), curcumin (curcuma). The citric bioflavonoids include hesperidin, quercitrin, rutin and tangeritin. Although initially it was suggested that the biological effects might depend on their anti-oxidant activity, cell cultures studies suggest that their biological effects result from the flavonoid ability to interact with different molecules along the cell growth and apoptosis signalling pathways. Also the flavonoid metabolites retain the ability to interact with the proteins from the signalling pathways. The involvement of flavonoids in cell signalling might be one of the factors responsible for their anti-cancerous, vascular and cardio-protective activities.

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