Bone and the balance of bone metabolism

The human skeleton provides many basic functions, including normal bone metabolism. Bone plays an important role in maintaining physiological homeostasis of the body. The balance between osteolysis and osteogenesis is important in bone metabolism. This process reflects the osteoblast and osteolytic cell function of coordination. RANKL/RANK/OPG pathway plays a central regulatory role in normal bone remodeling. RANKL/RANK/OPG regulates the axis of Bone, which is an important way to influence bone formation and dynamic equilibrium. In the process of tumor osteolytic bone metastasis and giant cell tumor (GCT) of bone, the expression of RANKL increases, which leads to excessive bone resorption. Denosumab can specifically block RANKL, thereby inhibiting the activity of osteoclasts and blocking the development of disease. Skeletal-related event (SRE) caused by bone metastases not only reduce patients’ physical function and quality of life, but also increase the risk of death. How to optimize the management of bone metastasis and relieve the pain caused by bone metastasis has become a hot spot in the field of cancer. Based on a series of clinical studies, Denosumab has been approved by the U. S. Food and Drug Administration for osteoporosis, solid tumor bone metastasis, GCT of bone, malignant tumor hypercalcemia and other fields. In China, Denosumab was indicated to treat GCT of bone, as well as SRE caused by solid tumor bone metastases and multiple myeloma.

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progenitors, like other hematopoietic cells, are released into circulation (7). Bone cells are osteoblasts embedded in the matrix, surrounded by collagen and inorganic salts. The cells related to osteolysis are mainly osteoclasts. Osteoclasts is derived from blood cells derived from macrophage precursors (hematopoietic precursors of the monocyte-macrophage lineage) and plays a role in bone remodeling by absorbing bone matrix. Normal bone metabolism requires a balance between osteogenesis and osteolysis. In the body, normal bone precisely undergoes a four-stage cycle of bone resorption, bone repair, bone formation and dormancy. Both osteoblast and osteoclast cell types are affected by a variety of hormones, inflammatory mediators and growth factors (8,9). An imbalance in the function of osteoblasts and osteoclasts can lead to bone abnormalities. An imbalance is characterized by an increase in bone mass (osteosclerosis) or a decrease (osteoporosis). We present the following article in accordance with the Narrative reporting checklist (available at http://dx.doi.org/10.21037/tbcr-20-69).

The discovery of OPG

In the 1990s, Amgen Inc. used cDNA to find anti-tumor drugs (10). In the course of the study, a protein was found to protect bone from osteolysis by osteoclasts, hence the name “Osteoprotegerin” (OPG) (11). In addition (12), OPG ligand (OPGL), which can bind to OPG, was screened by recombinant fusion technology, and a cytokine related to tumor necrosis factor (TNF), called RANKL (activator of nf-kb receptor activator ligand), was found. The receptor for RANKL was also identified in DC cells (DENDRITIC cell). RANK (a receptor activator of nuclear FACTOR-KAPPA B) was also found to be expressed in osteoclasts and their precursors. More coincidentally, the RANKL sequence is identical to the OPGL sequence. As a new secretary member of the Tumor necrosis factor receptor family, this new member lacks any obvious cell related signals, suggesting that it may play a role in the extracellular environment, transgenic mice expressing this secretory protein showed a general increase in bone mineral density (Ossification) and a decrease in osteoclasts. Recombinant OPG has a similar effect in normal mice, decreasing the extracellular environment, and preventing ovariectomy-related bone loss in rats, and OPG prevents osteoclast differentiation, it acts as a humoral regulator of bone resorption (13-15).

RANK is expressed on the surface of dendritic cells and t cells and hematopoietic precursors and is the receptor for RANKL. The combination of RANK and RANKL may regulate dendritic cell function and T cell activation. RANK maps to human genome 18q22.1, and RANKL maps to human genome 13q14, the first TNFR and TNF ligand family members to map to these regions (16).

Osteoprotegerin, expressed in osteoblasts, can bind to and inhibit the function of RANKL, thus preventing bone loss and increasing osteogenesis. Many diseases are characterized by an imbalance of bone metabolism, both in local and in systemic situation. In the pathophysiological process, an increase in osteolysis is the result of the overproduction of RANK-activated osteoclast-like giant cells. Pathological overproduction of RANKL can be caused by a number of factors, such as direct tumor secretion. The stromal cells in giant cell tumor (GCT) of bone can produce excessive RANKL. Excess RANKL can also be caused by other factors that stimulate osteoblast secretion, such as endocrine, infection, bone disease, bone metastases, myeloma, and so on. However, taking measures to neutralize the excess RANKL does not kill the tumor, but inhibits absorption, upregulates OPG, increases osteogenesis, and reduces and repairs bone destruction.

A large number of OPG variants have been explored in preclinical models, among which the form of OPG monomer, Polyanethylene glycol, and RANK-Fc fusion of RANK and antibody FC segments is only in the preclinical stage (15). But basic research has shown great commercial value for researchers, and the development of RANKL inhibitors is imperative. At the clinical stage, the modified version of the recombinant OPG combines the active region of OPG with the c-terminal of FC, which is the first type of OPG (Fc-OPG) in human trials. The glycosylated OPG-Fc (AMGN-0007) combines the active region of OPG with the n-terminal of FC, the half-life is about ten times longer and three to ten times more potent than the Fc-OPG (17-19). Given the potential safety risks of inducing an immune response to OPG in an individual population, Amgen stopped developing OPG-fc and turned to a method of producing RANKL.

Development of human antibody to RANKL (denosumab)

In a major advance in antibody therapy, the transgenic mice provided full humanized monoclonal antibody that could be produced quickly and with high affinity, the antibody subtypes of IgG2 have few deleterious molecular effects on cells expressing RANKL (such as ADCC and
CDC, antibody-dependent cell-mediated Cytotoxicity, and complement-dependent cytotoxicity) (20). Natural IgG2 antibodies differ from IgG1 and IgG4 in their hinge disulfide bonds, are highly susceptible to Papain and lose their potency through hydrolysis, and Amgen has a well-developed patented technique for modifying the hinge region of IgG2 antibodies, the modified IgG2 antibody reduces isomer differences and increases resistance to Papain. On the basis of this technique, a new humanized monoclonal antibody was born, with a molecular weight similar to that of the recombinant OPG-Fc, which can be combined with soluble or membrane bound human RANKL, denosumab was more specific than human OPG-Fc and prolonged the half-life of denosumab significantly (15,21,22).

During the developing process of RANKL inhibitor as a therapeutic candidate, the scientist at Immunex Company pursued a conceptual and design strategy similar to that used for the development of etanercept. However, following repeated dosing of human RANKFc in primates, activating autoantibody against RANK were detected that led to hypercalcaemia. This highlighted the potential risk of an immune response to endogenous RANK in patients, and RANKFc development was discontinued. An improved version of recombinant OPG was produced in bacteria as an amino-terminal IgG Fc region fused to the ligand binding domain, and was the first version of OPG that was tested in humans (Fc-OPG). A superior recombinant OPG-Fc fusion protein (a C-terminal immunoglobulin Fc fusion protein comprising residues 22–194) known as AMGN-0007 was produced in mammalian cell cultures and was found to be glycosylated. Upon testing in humans, its potency was determined to be at least 20 times better than the bacterial Fc-OPG protein. Denosumab (known at the time as AMG 162) is a human IgG2κ, and demonstrated in vitro neutralizing activity; it also had a similar molecular mass to recombinant OPG-Fc fusion proteins but a modestly lower affinity for human RANKL (10). The mechanism of Denosumab could be illustrated by Figure 1.

**Denosumab treatment for bone metastasis**

Based on a series of clinical studies, Denosumab has been approved by the U. S. Food and Drug Administration for osteoporosis, solid tumor bone metastasis, GCT of bone, malignant tumor hypercalcemia and other fields. In China, Denosumab is also approved for bone metastases. The incidence of bone metastases is as high as 65–75% in patients with advanced breast cancer (23). The efficacy of denosumab as a bone-modifying agent in the treatment of Bone metastases from advanced breast cancer has been demonstrated in clinical studies.

The 136 study was a multicenter phase III clinical trial involving 2,046 patients with bone metastases from advanced breast cancer and compared the efficacy of denosumab
with zoledronic acid (24). The results showed that the time between denosumab and zoledronic acid to first SRE was significantly longer (less than 26.4 months), and the risk of multiple SRE was reduced by 23% during the study period. Denosumab group was also significantly better than zoledronic acid group in delaying the onset of moderate to severe pain by 3.9 months. The improvement in quality of life was also better in the denosumab group, with HRQoL improving 10% more patients than zoledronic acid. A Phase III study of Denosumab versus zoledronic acid in the treatment of advanced solid tumors and multiple myeloma included 1,597 patients, including 811 with bone metastases from lung cancer (25). In the Lung Cancer Subgroup, denosumab significantly prolonged survival by 1.2 months (8.9 versus 7.7 months) compared with zoledronic acid and reduced the risk of death by 20%. Among the overall lung cancer patients, NSCLC, especially squamous cell carcinoma, had the most significant survival benefit and a 32% reduction in mortality risk. In addition, denosumab significantly delayed the median time of pain exacerbation (8.2 versus 4.8 months) compared with zoledronic acid in baseline pain-free or mild-pain solid tumor patients.

Denosumab treatment for GCT of bone

The exact pathogenesis of GCT of bone is unclear. The imaging findings of GCT of bone were osteolysis. It was suggested that the osteolysis process of GCT was dependent on the combination of RANKL (receptor activator of nuclear κB-ligand) and RANK (26,27). There are two kinds of cells in the pathological morphology of GCT of bone: monocytes and osteoclast-like multinucleated giant cells. There are two types of monocytes: spindle stromal cells and macrophage cells. Spindle stromal cells (fusiform stromal cells) are tumor cells of GCT of bone. Macrophage are precursors of osteoclast like cells that fuse together to form osteoclast like multinucleated giant cells. Osteoclast-like giant cells express RANK, while spindle stromal cells express RANKL and RANKL-RANK, which activate RANK-RANKL pathway and induce osteolysis.

RANKL as a key factor in the activation of osteoclast functional pathway has aroused great interest of scientists. In the research of RANKL-RANK-OPG pathway, Amgen has done a lot of work. Amgen eventually developed a humanized anti RANKL monoclonal antibody. Denosumab can competitively bind RANKL secreted by stromal cells, thus significantly reducing or eliminating the recruitment of osteoclast-like giant cells, thus blocking the activity of osteoclast-like giant cells, avoiding the osteolysis process, and increasing the formation of new bone, to slow the progression of the tumor.

Summary

Bone-modifying agents are the main bone-targeting agents for the treatment of bone metastases, including bisphosphonates and Denosumab, which can control the development of bone metastases to a great extent. Denosumab is the first precisely targeted RANKL inhibitor that blocks the binding of RANK to RANKL. Due to the unique mechanism of action of Denosumab, the initial use may bring more benefits to patients.

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Footnote

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