Humoral Regulation of Osteoclasts and Their Role in Bone Resorption

Osteoklastların Humoral Regülasyonu ve Kemiğin Rezorpsiyonundaki Rolleri

Hatice Sebila Dökmetaş

Cumhuriyet University Medical Faculty, Department of Endocrinology and Metabolism, Sivas, Turkey

Abstract

Osteoclasts are derived from the macrophage haematopoietic lineage, resemble monocyte-like phagocytic cells, and are involved in bone resorption. The cells of the bone and the immune system communicate by cytokines and growth factors. The discovery of the receptor activator of nuclear factor kappa B (RANK) signalling pathway in osteoclasts provides a deeper understanding of osteoclastogenesis, mechanisms of the activation of bone resorption, and how bone structure and mass are affected by hormones. *Turk Jem 2008; 12: 12-7*
Key words: Osteoclast differentiation, osteoclast activation, bone resorption, RANK signalling

Özet

Osteoklastlar, hematopoetik makrofaj dizisinden kaynaklanırlar. Monositik fagositoz yapan hücreleri andırırlar. Kemik ve immün sistem hücreleri, sitokinler ve büyüme faktörleri aracılığıyla iletişim kurarlar. "Nuclear factor kappa B" (RANK) sinyal iletisinin reseptör aktivatörünün osteoklastlarda keşfi, osteoklastogenezin kemik resorpsiyonunu uyaran mekanizmaların ve kemik yapı kitlesini hormonlardan nasıl etkilendiğinin daha iyi anlaşılmasına yol açmıştır. *Turk Jem 2008; 12: 12-7*

Anahtar kelimeler: Osteoklost farklılaşması, osteoklast aktivasyonu, kemik resorbsiyonu, RANK sinyali

Introduction

The bone is a dynamic organ in life; it is continuously molded, shaped, and repaired. The microstructure of the bone is organized to provide maximal strength by minimal mass to serve the physiological needs of an organism. Bone remodelling is a process that involves continuous renewal and daily increases or decreases in bone. Bone remodelling includes two close and equal procedures: bone break-down (resorption) and bone building (formation). The phases of bone remodelling in sequential order include activation, resorption, reversal, formation and resting.

Osteoclasts

Osteoclasts are extremely large multinucleated cells whose primary function is the resorption of bone and cartilage tissue. Bones contain 2-3 osteoclasts/mm^3. Osteoclasts are derived from the macrophage haematopoietic lineage, resemble monocyte-like phagocytic cells, and function like bone-specific macrophages. The multinuclear structure is one of the important features of these usually acidophilic stained cells, and one osteoclast can contain an average of 11-15 nuclei (ranging from 2-100) (1). Osteoclasts are located on endosteal bone surfaces within the resorption cavity, also known as Howship's lacunae. Each lacunae usually contains 2 osteoclasts, but this number can be as high as 5. The cytoplasm of the osteoclast cell contains multiple vacuoles, lysosomes, endoplasmic reticuli, and mitochondria, as well as an extensive golgi complex. The mean half-life of an osteoclast is approximately 2 weeks (1-4).

The characteristic ruffled border that forms at the site where the osteoclast is attached to bone matrix is a convolution of the plasma membrane with many long cytoplasmic processes (Figure 1). These structures, together with the contractile proteins surrounding it, maintain a more effective attachment of the osteoclast to the surface in order to carry out and facilitate the physiological activities. The plasma membrane is closely apposed to the bone

Address for Correspondence: Hatice Sebila Dökmetaş, Cumhuriyet University Medical Faculty, Department of Endocrinology and Metabolism, Sivas, Turkey
E-mail: sdokmetas@cumhuriyet.edu.tr

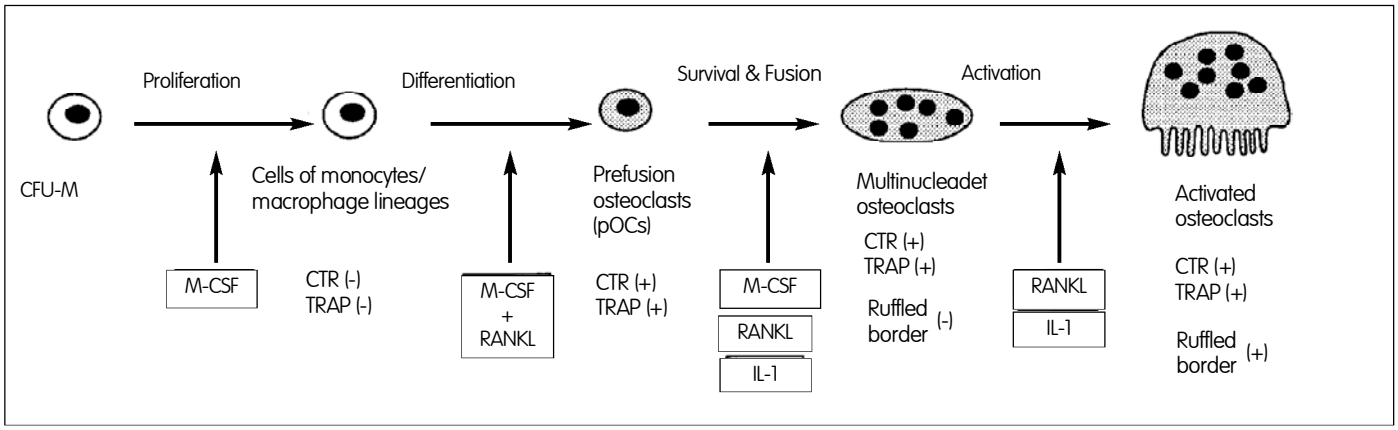

Figure 1. Osteoclast differentiation and activation (7)

surface with an adjacent organelle-free area, known as the clear zone. The clear zone contains abundant actin-like microfilaments which form podosomes. There are also various markers expressed on osteoclasts such as tartrate resistant acid phosphatase (TRAP), the calcitonin receptor (CTR), and the beta 3 integrin 7 receptor (3,5).

Bone Remodelling (Resorption and Formation)

Bone remodelling lasts for a lifetime, and is important not only for bone growth but also for the maintenance of normal bone structure. The procedure begins with the resorption of one part of the bone via osteoclasts, followed by new bone formation via osteoblasts.

The bone resorption phase begins with the attachment of the brush border to the bone surface. Hydrogen pumps at the brush border of osteoclasts produce an acidic environment, where the pH is about 3.5-4.0 in this area. Osteoclasts adhere to the bone matrix and then lytic enzymes such as TRAP and pro-cathepsin K (CATK) are released into Howship's lacunae and degrade extracellular components. Osteoclasts erode the underlying bone during this process, breaking down both the mineral and organic matrix of bone. As a result, collagen fragments and degradation products such as soluble calcium and phosphate are released into the circulation (Figure 2) (2-7).

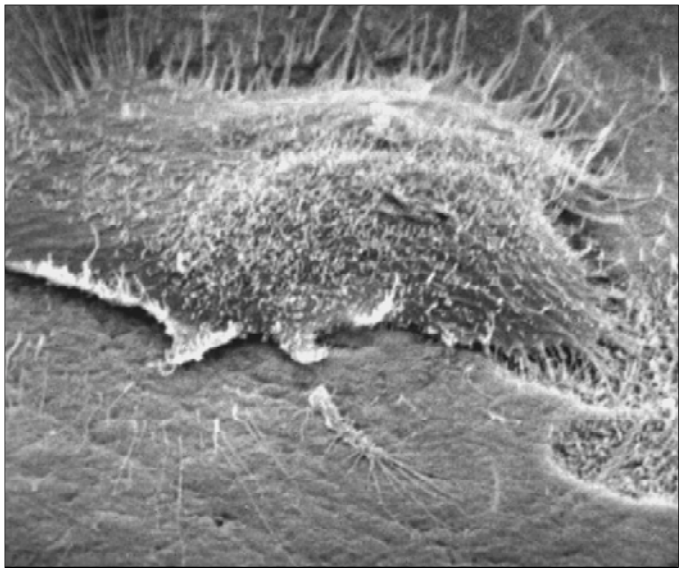

Figure 2. The appearence of osteoclast during resorption

Growth factors are in close relation with both the bone cells and the immune system via membrane-soluble and/or membrane-bound cytokines. Osteoblast lineage cells control osteoclast formation, activation, and bone resorption by local stimulatory mechanisms. Both T and B lymphocytes produce the activators and inhibitors of osteoclasts (6).

As bone growth is accomplished, there is always less bone formation than resorption in each cycle, which is known as an imbalance in remodelling. Any stimulus that increases bone remodelling results in an increase in the ratio of bone resorption to bone formation (5,6). Adult skeletal diseases are usually caused by the disturbance of the balance in favor of the increase in osteoclastic activity and resorption (8). A local coupling factor linking bone resorption to subsequent formation in the bone multicellular unit (BMU) has long been proposed as the key regulator of the bone remodelling process, but has never been identified. Growth factors that are derived from osteoblasts and released from the bone matrix during resorption take part in these coupling mechanisms (6).

Bone formation is regulated as positive during growth and negative during aging. This procedure occurs in the BMU. Parathyroid hormone (PTH) and gonadal steroids play a role in bone remodelling, but the actual regulation is directed by many cytokines and growth factors produced by bone cells and cells of the immune system (6). 1,25-dihydroxy vitamin D, PTH, and tumor necrosis factor (TNF) are some of the systemic factors that influence the formation of the osteoclast cell lineage. Cytokines and interleukins can also influence cell growth. Membrane-bound receptor activator of nuclear factor kappa B (RANK), RANK-ligand (RANKL), and macrophage colony stimulating factor (M-CSF) play important roles in osteoclast differentiation (6,9,10). Close proximity between osteoblastic lineage and haematopoietic cells is required for RANKL and M-CSF to bind to their respective receptors [receptor-activator of NF-κB (RANK) and the M-CSF receptor (c-fms)]. These receptors are expressed by monocyte and macrophage lineage cells. The binding of

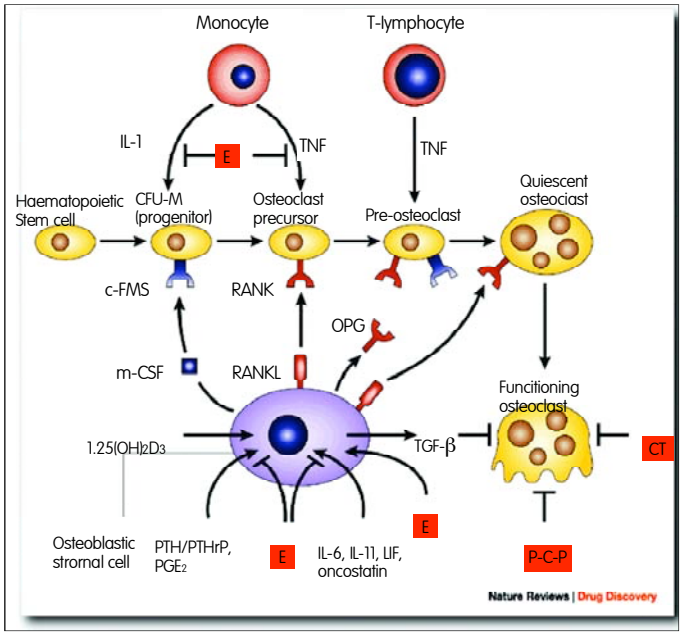

Figure 3. Differentiation and activation of osteoclasts

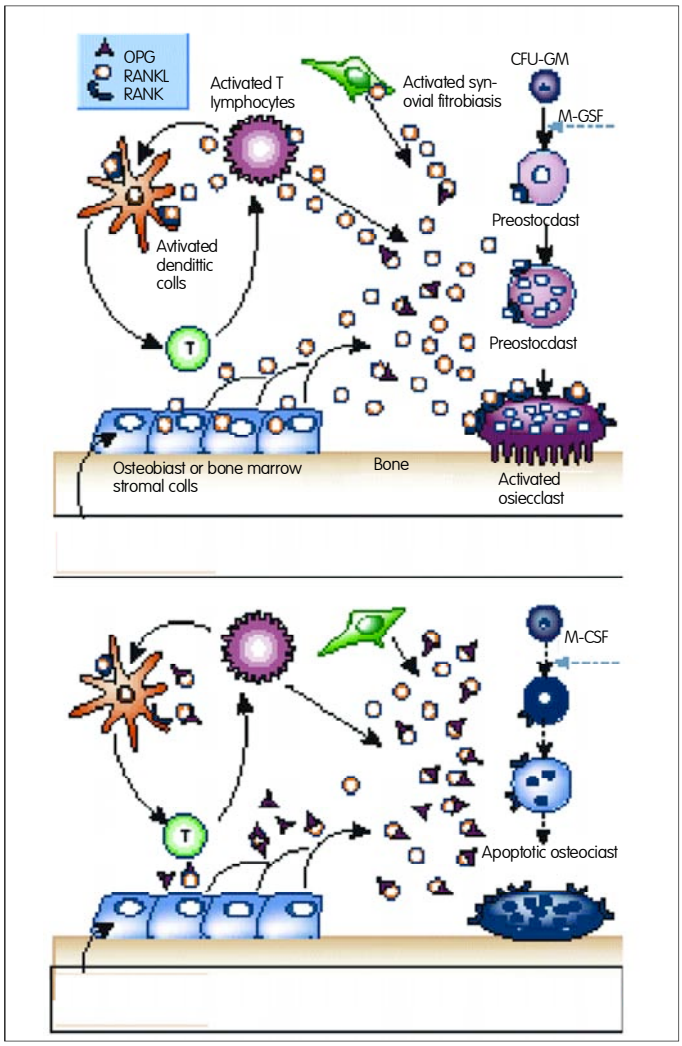

Figure 4. Differentiaton and activation of osteoclasts (11)

these ligands and receptors is also regulated by a secreted decoy receptor of RANKL, osteoprotegerin (OPG), which functions as a paracrine inhibitor of osteoclast formation (6,9,11). While M-CSF inhibits osteoclast maturation at high concentrations, it maintains the formation and survival of these cells at suitable concentrations. However, M-CSF itself is not sufficient for the formation of osteoclasts. Instead, certain local stimuli are required to direct progenitor cells to an osteoclastic cell lineage (9).

RANK/RANKL Pathway

The discovery of the RANK signalling pathway in osteoclasts provides a deeper understanding of osteoclastogenesis, mechanisms of the activation of bone resorption, and how bone structure and mass are affected by hormones. The TNF receptor (TNFR)/TNF-like proteins, as well as OPG, RANK and various biological factors such as RANKL, take part in osteoclast differentiation and activation (12). Tight contact between the stromal and bone marrow cells is mandatory for osteoclastogenesis, and factors that are released from stromal cells are thought to stimulate this process. M-CSF, RANKL, TRAP and the transcription factor known as nuclear factor of activated T cells 2 (NFAT2), can induce the genes needed for the development of mature osteoclast lineage. These genes include TRAP, CATK, CTR, and beta 3 integrin 7 (9,11).

RANKL is a TNF-associated surface protein. Its previous names were "osteoclast differentiation factor (ODF)", "TNF-related activation-induced cytokine (TRANCE)" and "OPG-Ligand". Its major sources are stromal cells, osteoblasts and activated T cells. In fact, the activation of T cells results in osteoclastogenesis and an increase in resorption (13). RANKL binds to the signal receptor RANK, which is a type II transmembrane protein expressed on the surface of pre-osteoclasts and mature osteoclasts.

OPG is a TNFR-related soluble protein which lacks transmembrane domains. Its sources are osteoblasts and bone stromal cells. OPG is also able to bind to RANKL, however it functions as a decoy receptor by inhibiting the RANK-RANKL connection (Figure 4) (4,5,7,9-11). Therefore, OPG blocks osteoclast resorption and osteoclast differentiation. OPG was also shown to regulate bone density and mass, as well as inhibit pathological bone resorption in animals. Excess OPG leads to hardening or increased bone density (osteopetrosis), while OPG deficiency leads to weakening or reduced bone density (osteoporosis) (11,14).

RANKL and OPG regulate bone resorption and coordinate the balance of high or low bone mineral density. Humoral factors like estrogen decrease the number of activated osteoclasts by increasing OPG expression and/or decreasing RANKL expression. As a result, bone resorption decreases and bone density increases (4,5,7,9-11). The ratio of RANKL / OPG is important and has a critical role in the pathogenesis of bone diseases due to bone resorption. Osteoclast activation increases as this ratio increases, and decreases as this ratio decreases (11). Therefore, RANKL is a key cytokine in osteoclastogenesis and bone resorption (12,15,16).

Certain hormones, cytokines and humoral factors are produced in distant organs but affect calcium homeostasis and bone density locally by inducing RANKL expression in bone cells (Table 1).

Table 1. The regulation of OPG, RANKL, and RANK (21)

OPG		RANKL		RANK
Bone morphogenetic protein-2	+	Autocrine motility factor	+	Unresponsive to most osteotropic agents, expression stable
Bone morphogenetic protein-7	+	Bone morphogenetic protein-7	+	
Calcium	+	Calcium	+	
1,25(OH)2 vitamin D3	+	Cyclosporine A	+	
17β-Estradiol	+	Dexamethasone	+	
IL-1	+	1,25 (OH)2 vitamin D3	+	
TGFβ	+	Fibroblast growth factor	+	
TNFα and β	+	Indian hedgehog	+	
Vasoactive intestinal peptide	+	IL-1	+	
		IL-6	+	
Cyclosporine A	-	IL-11	+	
Dexamethasone	-	Oncostatin M	+	
1,25(OH)2 vitamin D3	-	PGE2	+	
PGE_2	-	PTH	+	
PTH	-	Rapamycin	+	
Rapamycin	-	Tacrolimus	+	
Tacrolimus	-	TNF	+	
		M-CSF	+	
		Inhibin	-	
		TGFβ	-	
		Vasoactive intestinal peptide	-	

+, increase in expression; -, decrease in expression

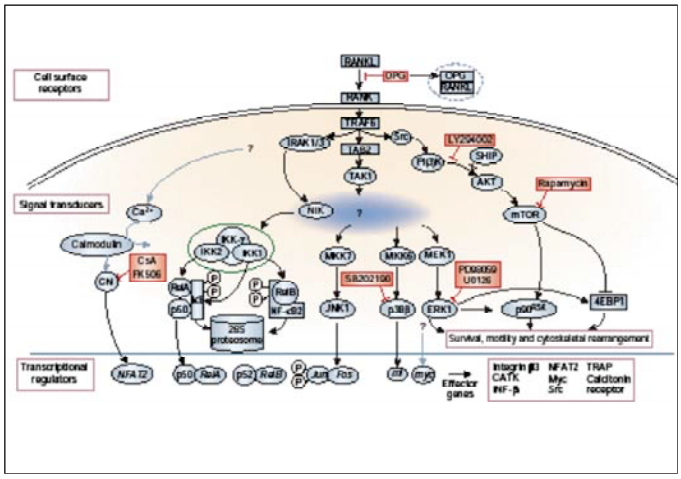

Figure 5. The RANK signalling pathway (11)

These hormones and factors can stimulate bone resorption by inducing RANKL expression in osteogenetic stromal cells in vivo. For example, most of the calcitropic hormones and pre-resorptive cytokines have been shown to upregulate the mRNA expression of RANKL (16,17). RANK deficient mice are resistant to the bone resorption related to TNF-α interleukin(IL)-1β 1,25-dihydroxy vitamin D, and PTH-related peptide (PTHrP), which constitute the most important calcitropic factors (9,11,12,14). Furthermore, RANKL expression by osteoblasts also coordinates bone remodelling (5,17).

RANK signalling is mediated by cytoplasmic factors. At least 5 different signalling cascades are induced by osteoclastogenesis and activation: inhibitor of NF-κB kinase (IKK), c-Jun N-terminal kinase (JNK), p38, extracellular signal-regulated kinase (ERK) and Src pathways. The first key step in RANK signalling is the binding of RANK with cytoplasmic factors that are part of the TNF receptor associated factor family (TRAF) in the cytoplasmic area. TRAF2, 5 and 6 have all been shown to bind to RANK, and of these factors, mutations in TRAF6 can lead to osteopetrosis due to the absence of osteoclast activation. Moreover, TRAF6 is a key regulator in gathering the signal proteins which direct the gene expression necessary for the differentiation and activation of osteoclasts (9,11,12,18).

Cytokines and hormones regulate the lifecyle of osteoclasts, as well as their the successful participation in bone resorption (2,3,9,11). RANKL and IL-1 increase the life-time of osteoclasts in vitro and in vivo (3). The crucial role of the RANKL/RANK/OPG signalling pathways in regulating bone metabolism is underscored by recent findings. Genetic mutations that activate RANK or inhibit the RANKL binding properties of OPG in humans are associated with familial forms of hyperphosphatasia and bone abnormalities. Several mutations in TNFRSF11B, the gene encoding OPG, have been found to be associated with idiopathic hyperphosphatasia (also known as Juvenile Paget's disease, which is an autosomal recessive bone disease characterized by deformities of long bones and the spine [kyphosis]) in humans (11). The

observations that mutations in the genes encoding RANK and OPG cause bone diseases of such striking severity in humans suggest that the inhibition of RANKL signalling may be a viable therapeutic strategy for the treatment of diseases where excessive bone resorption or remodelling prevail (6,9,11).

There are many control points present in RANK signalling that either stimulate or inhibit osteoclastogenesis (Figure 5). Activation of osteoclast surface receptors by IL-1, c-fms, TNF-α PGE_2 and transforming growth factor (TGF)-α may promote osteoclastogenesis in vitro and stimulates bone resorption in vivo. Both the IL-1 receptor and TNFR1 provide a signal to TRAF6, and activation of these receptors leads to a synergistic effect in TRAF6 activation (19). Activation of c-fms and TGF- also upregulates the components of the pathway including RANK levels (8,10). OPG negatively controls RANK signalling both in vivo and in vitro (9,11,17). Additionally, the chemical inhibitors of meiosis-specific serine/threonine-protein kinase (MEK1) and mammalian target of rapamycin (mTOR) increase osteoclastogenesis in vitro, which suggests that the activation of ERK and Scr pathways negatively regulate osteoclastogenesis (9,11).

There is some evidence about the presence of feedback mechanisms to inhibit the RANK signalling pathway. Induction of osteoclastogenesis by RANKL induces interferon (IFN)-γ secretion and this down regulates c-Fos expression which has a critical role in osteoclast development by an autocrine mechanism. INF-γ induces a negative effect on this pathway. Binding of IFN-γ to its own receptor leads to TRAF6 degradation and inhibits osteoclastogenesis in vitro (9,11,18). IL-4 also negatively regulates osteoclastogenesis, and signal transducer and activator of transcription (STAT) controls this process within the osteoclast (11).

NF-κB can be activated by either the alternative or the classical pathway (Figure 6). IKK, is involved in NF-κB activation by the classical pathway, where the p50 subunit of NF-κB is activated. On the other hand, IKK· is involved in NF-κB activation by the alternative pathway, where the p52 subunit of NF-κB is activated (20). The two most closely studied pathways are the activation of transcription factors NF-κB and activator protein-1 (AP-1), whose activities are rapidly induced following ligand binding. Targeted mutagenesis of the p50/p52 component of NF-κB, as well as the cFos component of AP-1, results in osteopetrosis due to inhibition of osteoclastogenesis. Activation of the NF-κB and AP-1 transcription factors can be induced by signalling cascades that are mediated by protein kinases, including IKK1/2 and JNK1, respectively (10,18). Recently, the mitogen-activated protein kinase (MAPK)-related TAK-1 has been detected in activated receptor complexes. TAK-1 is important in the activation of NF-κB and AP-1. MAPK-related kinase MKKK7 is required for JNK activation in these cells. However, it is not clear if TAK-1 acts directly on IKK1/2 or MKK7, or if other kinases mediate these events (11).

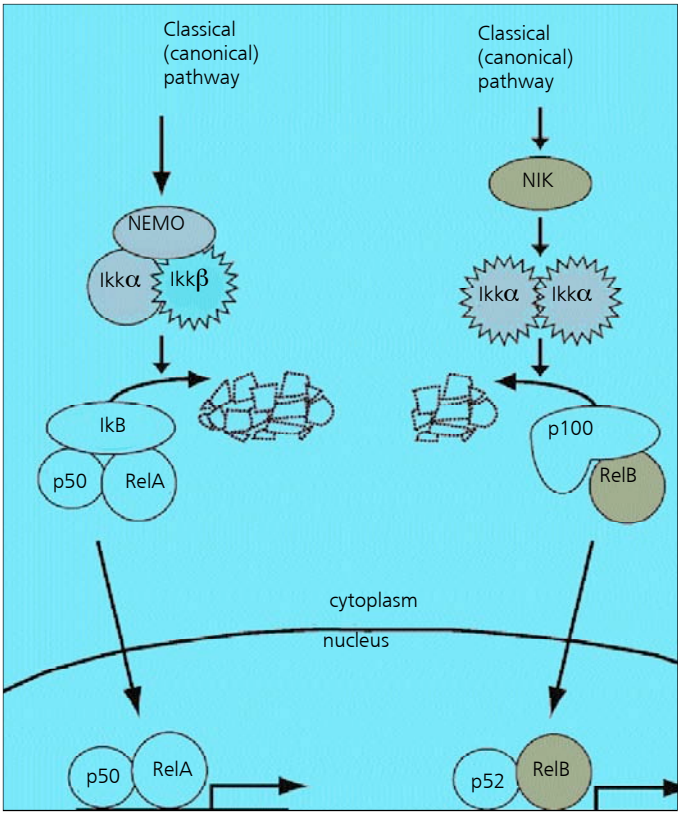

Figure 6. NF-κB can be activated by either the alternative or the classical pathway (21)

Summary

In summary, bone remodelling is a balance between formation (mediated by osteoblasts) and resorption (mediated by osteoclasts). The activation of osteoclasts is regulated by several cytokines and growth factors. Of note, the RANK/RANKL signalling pathway is one of the key pathways involved in osteoclastogenesis and resorption, and understanding this pathway provides insight into mechanisms of osteoclast regulation and activity.

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