Bone Homeostasis

The homeostasis of the skeleton is characterized by a strict balance of bone formation and bone resorption. These bone remodeling processes occur in tandem at site-specific locations due to the action of mainly two cell types. Mesenchyme-derived osteoblasts are the driving force for the formation of mineralized bone. Bone resorption (osteolysis) is caused by haematopoietic and myelomonocytic derived osteoclasts. These opposing cells are connected via paracrine cell signalling loops. A shifted balance in favor of multinucleated osteoclasts causes bone destruction as observed in pathological conditions such as autoimmune arthritis, periodontits, postmenopausal osteoporosis, Paget’s disease, Crohn’s disease and bone tumors.

RANK Signalling in Osteoclasts

RANKL

The receptor activator of nuclear factor xB ligand (RANKL [1]; OPGL [2]; TRANCE [3]; ODF [4]; TNSF11) has been identified as an essential cytokine for the formation and activation of osteoclasts. It is a type II membrane protein with close homology to the TNF superfamily ligand members TRAIL (TNSF10), Fasl (CD95L; CD178; TNSF6) and TNF-α (TNSF2). RANKL contains a C-terminal ligand-binding and a transmembrane domain, but exists as both in a membrane-bound and soluble form.

RANK

RANKL binds to receptor activator of NF-xB (RANK [1]; TRANCE-R; ODAR; TNSF11A), a cloned member of the TNF receptor superfamily sharing the highest homology with CD40 (40%). RANK is a type I membrane protein that associates at the cell surface and contains four extracellular cysteine-rich pseudorepeats.

OPG [Osteoprotegerin]

The effects of RANKL are physiologically counterbalanced by the decoy receptor osteoprotegerin (OPG [5]; OCIF [6]; TR1 [7]; FDCR-1 [8]; TNSF11B), a naturally occurring member of the

Product Highlights

PAb to TRAF6 (Bur 30)

ALX-210-028-R050

50 µl

From rabbit. IMMUNOGEN: Synthetic peptide corresponding to aa 451-469 (DRLAKFLAPPEAQRTIPVFPRNNWL) of human TRAF6 (tumor necrosis factor (TNF) receptor-associated factor 6). This sequence is completely conserved in mouse TRAF6. SPECIFICITY: Recognizes human and mouse TRAF6. Detects a band of ~65kDa by Western blot. Does not cross-react with other members of the TRAF family. APPLICATION: IHC (PS), WB.

Calcitriol

[1α,25-Dihydroxyvitamin D3]

ALX-460-032-C100

100 µg

1 mg

Osteoactive hormone that up-regulates RANKL level and down-regulates OPG level. Biologically active form of vitamin D3, in intestinal calcium transport and bone calcium resorption.

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For NEW total/RANKL ELISA KIT see backcover!
TNF receptor superfamily. OPG contains 4 cysteine-rich domains, two death domain homologs and a heparin binding site, but does not have a transmembrane domain. The OPG gene product is a 401 aa polypeptide, which is signal peptide-cleaved to 380 aa (44kDa), N-linked glycosylated (55kDa), and secreted as a disulfide-linked 110kDa homodimer. Dimerization is unusual among TFRSF proteins, which typically associate as trimers. In vivo administration of OPG has been shown to increase bone mineral density and bone volume associated with a decrease in the number of active osteoclasts [5]. OPG binds also to the TNF superfamily ligand TRAIL.

**Signalling Pathways**

In bone, RANKL is produced primarily by osteoblasts and bone marrow stromal cells. Binding of RANKL and macrophage/monoctye-colony forming factor (M-CSF) to their receptors (RANK and c-fms) on osteoclast precursors lead to osteoclast formation, while the binding of RANKL to its receptor (RANK) on mature osteoclasts triggers their activation and survival. *Six key signalling pathways are activated upon binding to RANK: Akt/PPK (protein kinase B), Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), p38, nuclear factor κB (NF-κB) and nuclear factor of activated T cell 1 (NF-ATc1).* One member of the tumor necrosis factor receptor associated factor (TRAF) family, TRAF6, binds to one of three cytoplasmic domains of RANK upon RANKL association, thereby activating the three mitogen activated protein kinase (MAPK) pathways (JNK, ERK and p38) and the NF-κB pathway. TRAF6 forms a complex with the scaffolding protein cIAP1 and phosphoinositide 3-kinase (PI3K) to activate the Akt/PPK pathway. Another signalling complex of TRAF6, TGF-β-activated kinase 1 (TAK1) and adapter protein TAB2 leads to the activation of the NF-κB and JNK pathways. Activation of p38 occurs upon its binding to TAB1 recruiting it to TRAF6/TAK1. The involvement of TRAF6 and the role of the cytoplasmic domains of RANK in the activation of the ERK pathway have yet to be defined. Transcription factor NF-ATc1 is a main regulator in osteoclastogenesis. NF-ATc1 is activated by RANKL and induces the expression of its own gene but is only fully activated if ITAM-mediated signals are present.

**Effectors on RANK Signalling (ITAM-mediated Signals and Interferons)**

RANK signalling is effected by further factors. Immunoreceptor tyrosine based activation motif (ITAM) is part of cytoplasmic domains of several transmembrane adapter molecules which link to immunoglobulin-like receptors. Two examples of these ITAM containing adapters are DNAX-activating protein 12 (DAP12) and Fc receptor common γ subunit (FcγRIγ) which have been shown to activate together calcium signalling, leading to the full activation of NFκB1 in osteoclast precursor cells [9]. While full activation of ITAMs requires signals from both the immunoreceptors and RANK signalling, the phosphorylating tyrosine kinase(s) activated by the RANKL signalling remain unknown.

RANKL induces interferon-β (IFN-β) but not interferon-α (IFN-α) expression in osteoclast precursor cells. IFN-β strongly inhibits the osteoclast differentiation by interfering with the RANKL-
induced expression of c-Fos. Activated T cells maintain bone homeostasis by counterbalancing the action of RANKL through production of IFN-γ. This cytokine induces rapid degradation of TRAF6 via signal transducer and activator of transcription 1 (STAT1) by proteasome activation and poly-ubiquitination.

Other Tumor Necrosis Factors (TNF-α, TRAIL and Fast)

As a major cytokine of inflammatory responses, TNF-α plays a role in many diseases, such as postmenopausal osteoporosis, rheumatoid arthritis and periodontitis. It suppresses the recruitment of osteoblasts from progenitor cells and inhibits the expression of matrix protein genes necessary for bone mineralization. It also increases the production of interleukin-6 (IL-6) and M-CSF by osteoblasts, thereby indirectly promoting differentiation of osteoclasts and enhancing bone resorption. TNF-α can also modulate osteoclast formation and function by enhancing the expression of RANKL by osteoblasts andstromal cells. TNF-α is suggested to induce vitamin D resistance. The active metabolite of vitamin D3, calcitriol (1,25(OH)2D3), contributes to skeletal health by stimulating intestinal calcium absorption and also by directly regulating bone cell gene transcription.

Osteosclerosis life span may be a keystone to bone remodeling. Because FAST1, TRAIL and their receptors Fas (TNFSF6) and TRAIL-R1 to -R4 (TNFSF10 to 10D) are well known as cellular regulators of apoptosis, they may also be of interest in bone research. However, the precise role of these modulators in bone remodeling need further investigation.

Osteoporosis and Obesity/Adipokines

Leptin

Leptin, a 16kDa cytokine like hormone secreted by adipocytes is known to reduce appetite, but has now also emerged as a significant factor in the regulation of bone mass. In one of two alternate pathways involving a hypoalumus relay leptin gets transported to the central nervous system (CNS). Here it binds to neurons of the ventral hypothalamus and stimulates the hypothalamic-sympathetic nervous system (SNS) to release noradrenaline which binds to adrenergic receptors on osteoblasts inhibiting their bone forming function. Recent studies describe a second pathway in which leptin may act directly as a local mediator binding to leptin receptors expressed by osteoblasts, thereby inducing proliferation and differentiation [10, 11].

Adiponectin

30kDa adipocyte complement-related protein (adiponectin; ACRP30), is another adipokine reported to be negatively associated with clinical manifestations as obesity, diabetes and cardiovascular diseases. Adiponectin was thought to be adipocyte-specific. Recently the expression of adiponectin and its putative receptors AdipoR1 and AdipoR2 in osteoblasts has been shown and an activation via p38/JNK proposed [12, 13]. However, the biological role and molecular mechanisms of adiponectin in bone need further clarification.

References

Literature Overview:

Selected Latest Review Articles:

**Bone Research Proteins**

**RANKL (human):Fc (human) (rec.)**

**ALX-522-039-C050**

Produced in HEK 293 cells. The cysteine-rich region of human RANK (aa 29-215) is fused to the Fc portion of human IgG1. BIOLOGICAL ACTIVITY: Inhibits human rhRANKL-induced survival of dendritic cells and osteoclasts.

**Osteoprotegerin (human):Fc (human) (rec.)**

**ALX-522-007-C050**


**Latest Product Additions**

**Adiponectin Paralogs**

C1q tumor necrosis factor-related proteins (CTRPa) 1-7 are a new highly conserved family of adiponectin paralogs. A member of this family, CTRP2 (mouse) has been reported to rapidly induce phosphorylation of AMP-activated protein kinase, acetyl-CoA carboxylase, and mitogen-activated protein kinase in C2C12 myoblasts, which resulted in increased glycogen accumulation and fatty acid oxidation.

**NEW** 
Pab to CTRP2 (mouse) (AT102)

**ALX-210-923-C050**

From rabbit. IMMUNOGEN: Recombinant mouse CTRP2 (aa 26-260). SPECIFICITY: Recognizes mouse CTRP2. APPLICATION: IP, WB.

**NEW** 
Pab to CTRP7 (mouse) (AT103)

**ALX-210-924-C050**

From rabbit. IMMUNOGEN: Recombinant mouse CTRP7 (aa 18-290). SPECIFICITY: Recognizes mouse CTRP7. APPLICATION: IP, WB.