Arboleya L[1], Castañeda S[2]
1 Hospital Universitario Central de Asturias - Oviedo
2 Hospital Universitario de la Princesa - IIS-Princesa - Madrid

Osteoclasts: much more than bone remodelling cells

Correspondence: Luis Arboleya - Servicio de Reumatología - Hospital Universitario Central de Asturias - Avda. de Roma, s/n - 33011 Oviedo (Spain)
e-mail: arboleya@ser.es

Date of receipt: 07/06/2014
Date of acceptance: 07/10/2014

Summary
The osteoclast has been considered classically as a cell with the exclusive function of bone remodelling, with a gregarious behaviour.
However, advances which have been made in recent years have changed this concept drastically, and we now know that this multinuclear cell is subject to complex biological regulation, necessary for it to exert a multifunctional role of unknown dimensions.
In addition to its participation as the only cell capable of reabsorbing the calcified bone matrix, the osteoclast is one of the cellular elements effective in the immune system, a function still little-known but expected, given its belonging to the monocyte-macrophage lineage. Its role in other processes, both local, such as as a collaborative element in osteoformation and hematopoietic stem cell niche maintenance, and systemic, is also beginning to be understood.
In this review the most significant findings contributing to our understanding of the biology of the osteoclast are analysed, with an eminently practical content and an approach aimed at understanding the possible molecular targets which will allow a better therapeutic treatment of such important diseases as osteoporosis, arthritis or cancer.

Key words: *osteoclasts, osteoporosis, arthritis, RANKL.*

Introduction

Osteoclasts (OCs), as the only cells capable of extracting the calcified bone matrix, are the protagonists in the delicate task of dissolving the crystals of calcium phosphate and digesting the collagen, by means of highly specialised structures[1]. Their pathogenic role in the induction of excessive bone resorption observed in pathological processes such as osteoporosis[2], arthritis[3], or cancer[4], is fundamental. The notable advances which have occurred since the start of this new century have allowed us to understand the intimate mechanisms which regulate the formation, activity and survival of OC, opening new possibilities for the design of drugs with more specific actions than those that already exist.

In recent years, the scientific effort dedicated to understanding the complex resorptive mechanisms has grown exponentially, with great advances being made through three main lines of research: 1) the study of a series of genetic diseases, relating the phenotypes observed to the dysfunction detected; 2) experimental studies based on the creation of animal models in which a determined gene is annulled or overexpressed; and 3) by obtaining precursors and mature cells in culture and analysing their responses to various stimuli. Taking into account the fundamental importance of OCs in the pathogeny of such significant diseases as arthritis, osteoporosis and cancer, along with the enormous quantity of information which has emerged in the last five years, we consider it necessary to carry out a review to update our knowledge in this important area of research.

General characteristics of osteoclasts

OCs are located on the internal surfaces of the Haversian canals of the cortical bone, in the trabeculae large than 200 microns and in the external walls of the bone, beneath the periosteum. Although potential precursors may be found in the peripheral blood, spleen and bone marrow, the mature cells are very rarely found away from the bone surfaces, except in pathological situations, such as in giant cell tumours. In the absence of the specific situation of high levels of remodelling, such as occurs at the metaphysis of the long bones during growth or in diseases such as primary hyperparathyroidism, OCs are scarce in the skeleton since they only comprise 1-2% of bone cells. They have a half-life of two weeks, and in normal conditions, after this period, undergo apoptosis[5].

In spite of their rarity in samples of non-decalcified tissue, their morphology is characteristic when activated, which enables them to be easily recognised as strongly polarised multinucleated structures, with a basal region for the interchange of external signals and a zone joined to the calcified matrix by a structure called the brush border. The OCs move, by means of podosomes, over the calcified surfaces, on which a single cell can form consecutively a number of Howship's lacunae. They have a number of immunohistochemical characteristics which facilitate their identification, among which are the expression of tartrate-resistant acid phosphatase (TRAP). Although TRAP mRNA has been identified in other tissues, such as the kidney, intestine and lung, as well as in activated macrophages, this enzyme continues to be an essential osteoclast marker whose expression appears very early, immediately before the mononuclear OC initiates the fusion mechanisms, increasing progressively through the different post-fusion stages until maturity is reached.

The OCs belong to the monocyte-dendritic-macrophage lineage, although, differently from other members of its progeny, it has the capacity to bond to bone by means of the $\alpha v\beta 3$ integrins, which are expressed in the surface of the podosomes and which have the property of interacting with the proteins of the matrix, such as osteopontin and vitronectin. Following the primary activation signal, the multinuclear OC is polarised and is stuck to the bone surface by means of specialised structure known as the brush border, at the ends of which are found the integrins which become bonded to the matrix producing a hermetic seal with the lacuna, an essential step for the interchange of ions and proteases necessary for proper bone resorption.

The basolateral zone of the membrane does not undergo significant morphological changes, but will play a role, which is poorly-understood, in cell communication and in the transport of ions. In the osteoblast cytoplasm there is a high level of carbonic anhydrase II activity which causes a dissociation of the cytosolic carbonic acid into protons (H^+) and bicarbonate ($HCO3^-$), the latter interchanged with chloride (Cl^-) by means of a specific channel, which allows the conservation of the intra-cellular isoelectric state. The proton is directed to the brush border, where a proton pump dependent on a specific ATPase (H^+-ATPase) transports it to the lacuna. In the vicinity of this pump is situated an ion channel (chloride 7 channel, ClC7) which is a simple ion interchanger which uses voltage gradient to obtain the energy necessary to transport them through the membrane. Specifically, this channel interchanges 2 Cl^- for 1H^+, and its function is highly important in the processes of lysosome acidification in general[6] and in bone resorption in particular.

The loss of function of the ClC7 is one of the most common causes of osteopetrosis[7] and is, together with the proton pump, an interesting therapeutic target[8], but limited, at the moment, due to the consequences of its extra-skeletal actions, above all, the risk of production of lysosomal diseases[9]. In the lacunae, through the union of these two ions, hydrochloric acid is formed, which acidifies the environment causing the hydroxyapatite to dissolve, liberating calcium and phosphate, while at the same time maintaining the cytoplasmic ionic charge in equilibrium. Lastly, through the lysosomes, a cysteine protease, cathepsin K, and a series of metalloproteases are secreted which, finally, cause the dissolution of the organic matrix. The resulting degradation products enter the OC by endocytosis and are transported to the basolateral region in vesicles rich in TRAP and released to the exterior by exocytosis.

Formation and activation of osteoclasts

The osteoblasts (OB) of mesenchymal origin reside, essentially, in the bone tissue and the adjacent bone marrow. However, the OCs and their precursors are a highly dynamic population, and the mechanisms which control their migration and arrival at the bone surfaces have recently emerged as essential elements of the homeostasis of the skeleton. OCs derive from hematopoietic stem cells, which will lead, through myeloid progenitors, to circulating monocytes and tissue macrophages[10]. The target organ will define the final characteristics of these cell populations, emitting different signals which will determine their different morphological and functional qualities: Küpffer cells in the liver, alveolar macrophages in the lungs, microglia in the central nervous system, histiocytes in the connective tissue, dendritic cells and macrophages in the lymphoid organs, and OCs in the bone. In spite of the fact that many of the properties of these differentiated myeloid cells, essentially their structure and function in the tissues, are known, there is still very little known of the intimate mechanisms which govern their differentiation and dynamics.

Migration of the precursors

Mononuclear lineage cells with the capability of differentiating into osteoclasts have been found in the bone marrow and in the bloodstream[11,12]. Although it is not known if there is a mononuclear precursor population specific to OCs, it is known that certain sub-classes of circulating monocytes and dendritic cells, as well as progenitor cells of monocyte-macrophage lineage resident in the bone marrow, have the capability of being transformed into OCs if they are subject to certain specific signals[13]. Using innovative fluorescence techniques which allow the visualisation of the behaviour of cells *in vivo*, Kotani et al. have recently shown that the mature OCs situated in the resorption surfaces come from the circulating monocytes which migrate to these regions of the bone where they undergo fusion, polarisation and development of the elements of the cytoskeleton which characterise active OCs[14].

The signals which attract the circulating precursor population towards the bone surfaces are starting to become understood, constituting an interesting group of molecules of potential therapeutic interest. These cells, which should express RANK in their membranes, become attracted to the bone marrow or the quiescent surfaces where, after receiving the RANKL signal, they are transformed into mature, polarised OCs with the characteristic cytoskeleton. This main signal comes from the mesenchymal cells of the bone marrow, from lining cells or from the osteocytes situated in the depths of the calcified matrix.

The RANKL signal is essential for the final activation of the OCs, although it is probably only executed in the target organ, there being signals which we could consider to be "anterior" which provoke the migration of the precursors from the circulation system. To date, various recruitment signals have been identified, notable among which is chemokine CXCL12, strongly expressed in stromal cells located in the perivascular regions of the bone marrow. The osteoclast precursors express the receptor of chemokine CXCR4, whose union with CXCL12 promotes the recruitment and survival of the OCs[15]. The CXCL12/CXCR4 axis has become a target of great interest in oncology[16,17] due to its key role in the migratory behaviour of tumour cells, although, taking into account the above, it is highly probable that it also participates in functions such as accelerated bone remodelling which occurs in postmenopausal osteoporosis, or in the different forms of bone destruction which characterise rheumatoid arthritis.

Another chemokine axis of interest is that featuring CXCL1 (fractalkine), expressed in osteoblasts, and its receptor, CX3CR1, expressed in OCs whose action could also be important in the recruitment of precursors[18]. Nevertheless, the design of small molecules with activity inhibitory to chemokines[19] is encountering a number of difficulties due to the toxicity caused by their poor specificity.

Another group of molecules with recruiting action are the bioactive sphingolipids. Known for their structural role in cell membranes, they have acquired additional importance due to their being precursors of molecules with a strong chemotactic capacity, such as sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P)[20,21]. The latter, with significant roles in the function and dynamics of other myeloid populations[22], does not appear to intervene in the migration of the OCs, with, to date, no receptors associated with these cells having been identified.

S1P is the product of the phosphorylation of sphingosine by two kinases, sphingosine-kinase 1 and 2, a reaction which is activated in response to a number of mediators which include various cytokines and hormones. After its synthesis it may be activated in the intracellular environment but also be released into the bloodstream, where it interacts with at least five G protein-coupled receptors, of whichS1PR1 and S1PR2 have been identified in osteoclast precursors[23,24]. After the bonding of S1P to its receptor, this is rapidly internalised in a way very similar to that which happens with the bonding of the ligand to CXCR4, and, at the present time, this is considered to be a highly significant factor in the dynamics of hematopoietic progenitor cells and in the traffic of immune cells between the lymphoid organs and the peripheral tissues. Its role in bone diseases is beginning to be understood, it having been observed that low concentrations of S1P are chemotactic for the osteoclast precursors, while high concentrations have the opposite effect. S1PR2-nul mice develop osteopetrosis, while in ovariectomised rats, the S1PR2 antagonist, JTE013, slows osteoporosis, reducing the number of OCs[24]. Contrarily, the ablation of osteoclast S1PR1 causes osteoporosis[25].

These facts suggest the existence of a fine control of osteoclast migration dependent on the gradient of S1P[26], which may be summarised as follows: in the bloodstream there is a high concentration of S1P, while in the bone tissue it is lower. The skeletal OCs, after the activation of the S1PR1, migrate towards the circulation system, while the activation of S1PR2 exerts an opposite effect, inducing migration in the opposite direction, with OCs accumulating in the bone. We are, therefore, looking at a molecular system of therapeutic interest[27-29], since the stimulus of S1PR1 or the blocking of S1PR2 causes an antiresorptive effect notable in murine models in, respectively, provoking the departure or slowing the arrival of OCs to the resorption sites.

Regulation of osteoclast differentiation

Osteoclast differentiation is a strongly regulated process whose study has been limited due to the necessity of using mixed cultures of osteoblasts and OCs to obtain mature cells[30]. Since the discovery of RANKL, the advance in the knowledge of these mechanisms has been enormous by making possible the culture of isolated osteoclast precursors in the presence of RANKL without the need for the interaction of other cells[31]. It is widely known that the mature OCs are the only cells in an organism capable of reabsorbing bone[32]. Nevertheless, to achieve the development of their complete resorptive mechanism the osteoclasts have to undergo a profound transformation after their arrival in the proximity of the mineralised surfaces, which starts with the initial intervention of M-CSF and the expression in its membrane of RANK (Figure 1). At present, the mechanism by which a sub-group of multipotential mononuclear precursors begin to express RANK in their membranes, and as a consequence, follow the path to differentiation as osteoclasts after being exposed to RANKL[33], is not known.

a) M-CSF signal

After the initial expression of PU-1, a transcription factor required for the generation of the progenitors of the lymphoid and granulocyte-macrophage series, which acts in the very early phases of myeloid differentiation, the expression of c-Fms occurs, the receptor of M-CSF which will characterise the population of the primitive osteoclast precursors[13,34]. After its union with the ligand, the c-Fms, as with other members of the super-family of tyrosine-kinase receptors to which it belongs, is phosphorylated and activated to ERK (extracellular signal-regulated kinase) through GRB-2 (growth factor receptor bound protein 2) and to AKT through Pl3K (phosphoinositide 3-kinase), provoking cell proliferation signals. In addition, through the activation of MITF (microphthalmia-associated transcription factor) the expression of Bcl-2 (anti-apoptotic B-cell leukaemia/lymphoma-associated gene 2) an essential factor for survival, is induced[35-38]. Lastly, the expression of RANK occurs in the membrane of the precursors, which will enable the action of RANKL on these cells and their final differentiation into mature OCs.

b) RANKL signal

RANK lacks intrinsic enzyme activity in its intercellular domain and needs to transduce the signal from the ligand through the recruitment of adaptor molecules, among them TRAF-6, GAB-2 (Grb-2-associated binder-2) and phospholipase C. The last two of these are not indispensable in the initial phase but are necessary in a subsequent amplification phase[39]. However, TRAF-6 is essential to activate the distal signal, in which NFkB, AP-1 and various MAPKs (mitogen-activated kinases), above all JNK (Jun N-terminal kinase), p38 and ERK, are involved.

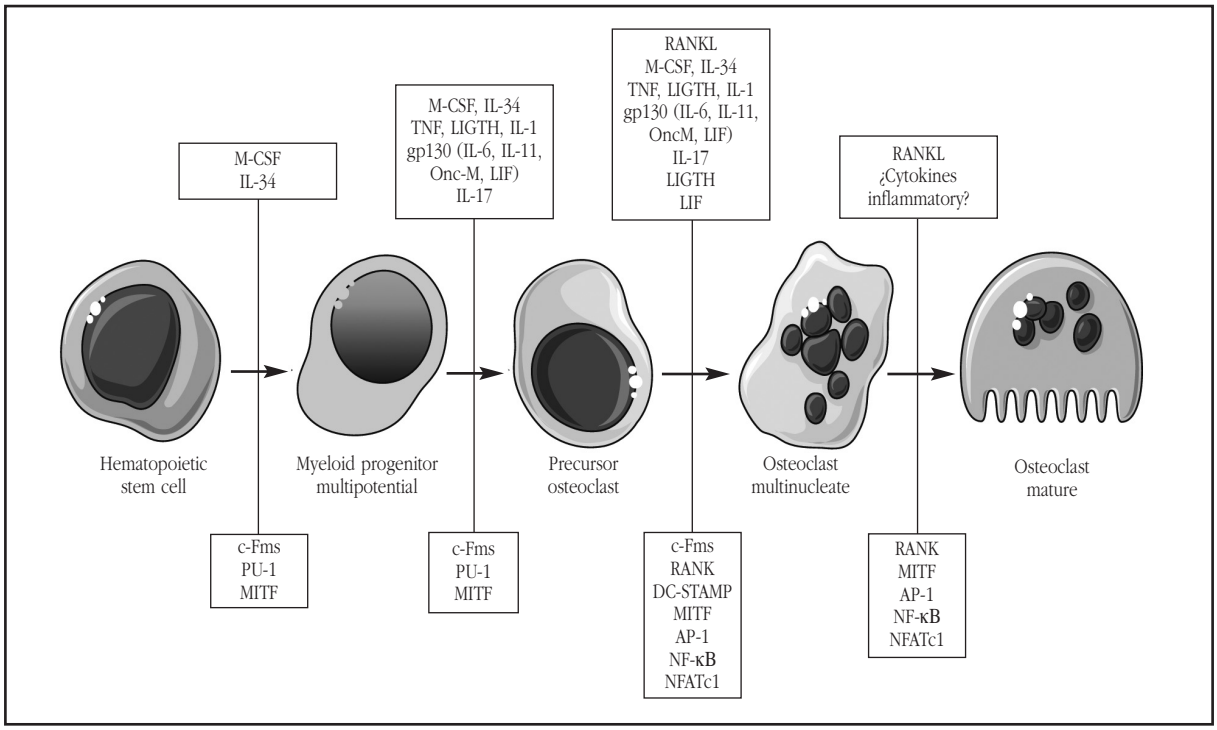
The activation of NF-κB is one of the earliest and most crucial molecular events which occur after the union of the ligand to RANK. NF-κB belongs to a family of dimeric transcription factors which, in the non-activated cell, stays captive in the cytoplasm due to being bonded with inhibitory proteins called IkB (inhibitors of κB kinase). The RANKL/RANK/TRAF6 signal provokes the proteolysis of these inhibitors, which allows the translation to the nucleus of free NFkB, where it bonds with DNA response elements, inducing the transcription of the target genes[40]. This intracellular signalling pathway participates in the regulation of various genes involved in immune and inflammatory responses, which produce cytokines such as IL-1, IL-2, IL-6, IL-7 and TNF, chemokines, interferons and anti-apoptotic proteins, such as BIRC2, BIRC3 and BCL2L1. In humans, the deregulation of NF-κB is associated with various diseases such as diabetes mellitus, Alzheimer's, autoimmune diseases, osteoporosis and arthrosis, and is a potential therapeutic target, partly limited by its non-specificity[41].

RANK also induces the activation of NFATc1 (nuclear factor of activated T-cells, cytoplasmic 1), currently considered to be the master regulator for osteoclast activation[42]. NFATc1 belongs to the family of NFAT transcription factors, identified initially in nuclear extracts of activated T-lymphocytes[43]. In subsequent studies it was shown that its role in osteoclast activation was significant when it was observed that the monocyte-macrophage precursor cells in bone marrow stimulated by RANKL had a selective and marked overexpression of NFATc1[44]. The activation of this factor is dependent on NFkB and c-Fms, probably in this order[45].

c) Co-stimulation and amplification of the RANKL signal

Coordinated with the RANKL signal other transduction pathways for inductor signals for NFATc1 have been observed in the OC (Figure 2), whose role could be decisive in pathological states[46]. At least two Ig-like receptors are known: OSCAR[47] (osteoclast-associated receptor) and TREM-2[48] (triggering receptor expressed in myeloid cells). Both are associated with adaptor proteins which contain ITAM (immunoreceptor tyrosine-based activation motifs) motifs such as DAP-12 (DNAX-activation protein 12) or FcRγ (Fc receptor common γsubunit). Although the ligand for these receptors is not known with any certainty

Figure 1. Maturation stages of the osteoclast. In the upper section are shown the principle cytokines involved, and in the lower section, the transcription factors and transmembrane proteins. The PU-1 and MITF expression is the initial event which characterises the population of myeloid precursors which will go on to differentiate into osteoclasts. These two transcription factors provoke the expression of the M-CSF receptor which, after its bonding with the ligand, induces the expression of RANK. This fact is definitive for the formation of the mature osteoclasts, after the cytoplasmic, but not nuclear, fusion, governed by DC-STAMP



MITF: microphthalmia-associated transcription factor; DC-STAMP: dendritic cell-specific transmembrane protein; LIF: leukemia inhibitory factor; Onc-M: oncostatin M.

(recently OSCAR has been associated with specific motifs expressed in fibrillar collagen)[49], when activated, the phosphorylation of the ITAMs by tyrosine-kinase occurs and, in collaboration with other molecules such as BLNK (B cell linker protein) and SLP76 (Src homology 2 domain-containing leukocyte protein of 76 kD), the activation of PLCγ2 is then provoked, contributing to the amplification of the RANK signal. It is not known whether these pathways are significant in physiological states, although in pathological situations such as osteoporosis, arthritis or cancer, it is highly probably that their over-activation contributes to the state of marked osteoclast stimulation which they exhibit[47-52].

NFATc1 is a regulator central to osteoclast activation, both in the sense of being a stimulator of the RANK signal and in the opposite sense, as a target for different molecules which inhibit its expression. In the positive sense, the expression of NFATc1 induced by RANK/NFkB/c-Fos is dependent on the signalling pathway p38. Other signals, coming from Ig-like receptors associated with adaptor factors such as FcRγ y DAP12, act in a coordinated way with the above signals through the transitory increase in intracellular levels of calcium, due to mechanisms not yet clarified which could also involve PLCγ2, which then activates calcineurin. This enzyme dephosphorylates the cytosolic NFATc1, which allows its translocation to the nucleus, where, in concert with PU.1 and MITF, it goes on to activate the promoter regions of various genes which code for molecules essential for osteoclast function such as cathepsin K, OSCAR, DC-STAMP, TRAP and V-ATPase-d2. In addition, there is an increase in its own synthesis through a process of auto-amplification described in 2005 by Asagiri et al.[45]. However, these secondary activation pathways of NFATc1 are dependent on the main pathway and, in the absence of RANKL, and no stimulus occurs in isolation from these receptors, leading to an absence of osteoclast activation[53].

To avoid unchecked osteoclast formation which would result from the NFATc1 pathway, there is a series of negative regulators which act on this factor, generally indirectly through the proximal signal[54]. Within the group of cytokines, IL-4 and IL-13, products of the Th2 cells, perform pleiotropic functions, among which is a powerful anti-osteoclast action which is executed in way which is dependent on STAT-6 (signal transducer and activator of transcription 6) with the final result being the expression of NFATc1. Other cytokines such as IL-10, IL-27 or IFN-γ inhibit the formation of OCs from their precursors or their activation, through mechanisms dependent on the RANK/NFkB/NFATc1 signal[55].

The activation of various TLRs (toll like receptors) reduces the rate of formation of mature OCs induced by RANKL through IFN-β-dependent mechanisms, although independent mechanisms have also been observed. On the other hand, the activation of TLRs is one of the most powerful inductors of inflammatory cytokines, such as TNF and IL-1, which act synergistically with RANKL in the production of inflammatory osteolysis in diseases such as rheumatoid arthritis or periodontal disease[56].

In brief, we may intuit that the TLRs, as key elements in the innate immune system, have an antagonistic role strongly dependent on context. On the one hand, by initiating the inflammatory response, the transformation of precursors into OCs would reduce, which would increase the pool of cells available for transformation into macrophages. However, in a more advanced stage, if their activation persisted in a sustained way, they would act as inductors for osteoclastogenesis, indirectly by means of inflammatory cytokines. The confirmation of this attractive hypothesis would constitute one more element to support the idea of the OCs' significant participation in the immune response.

There are other factors which inhibit the formation or activation of the OCs in addition to those already cited: cytokines such as TRAIL[57] (TNF-related apoptosis inducing ligand), IL-12 and IL-18[58], different intracellular signalling molecules such as SHIP1[59] (Src homology 2-containing inositol-5-phosphatase 1), NF-κB p100[60] and some components of the Notch pathway[61], various transcriptional repressors such as MafB (v-maf musculoaponeurotic fibrosarcoma oncogene family protein B)[62], C/EBPβ (CCAATenhancer-binding protein β)[63], IRF-8 (Interferon regulatory factor)[64], and BcL6 (B cell lymphoma)[65]. All these molecules are potential targets of therapeutic interest, but their detailed analysis is beyond the scope of this review.

d) Osteoclast activation pathways independent of RANKL

The RANKL signal is the most important osteoclast activation pathway and its annulment in murine models results in the complete disappearance of the OCs, which means that the role of pathways independent of activation appear, in theory, to be unimportant. However, in 2005 Kim et al. demonstrated that the presence of cofactors such as TGF-β, the hematopoietic precursors in mice null for RANKL, RANK and TRAF-6 would succeed in being differentiated into OCs[66]. It is evident that the interest in this topic is enormous, since there could be, at least in pathological circumstances, non-canonical osteoclast activation pathways which could be modulated to achieve different therapeutic responses to the complete annulment of OCs.

Within the TNF superfamily, given the structural homology between its members, various ligands and receptors have been investigated. One of the most interesting is LIGHT (also known as TNFSF14 and CD258). This type II transmembrane protein is expressed primarily in activated T-cells, NK cells, dendritic cells and macrophages, performing key biological functions in the innate and adaptive immune responses through the homeostasis, differentiation and activation of the T-lymphocytes[67]. It joins three receptors which share a structural similarity in their cytoplasmic stem: TNFRSF14/HVEM (herpes virus entry mediator), LT-βR (lymphotoxin β receptor) and DcR3 (decoy receptor 3)[68]. Although the role of LIGHT in bone resorption is not known, it has been observed that it causes a powerful osteoclastogenetic action independent of RANK and OPG, through AKT, NFkB and JNK in human and murine monocytes, using TRAF-2 and TRAF-5. Its function in bone diseases has not been clarified, but it is, without a doubt, an interesting target of potential therapeutic interest[69,70].

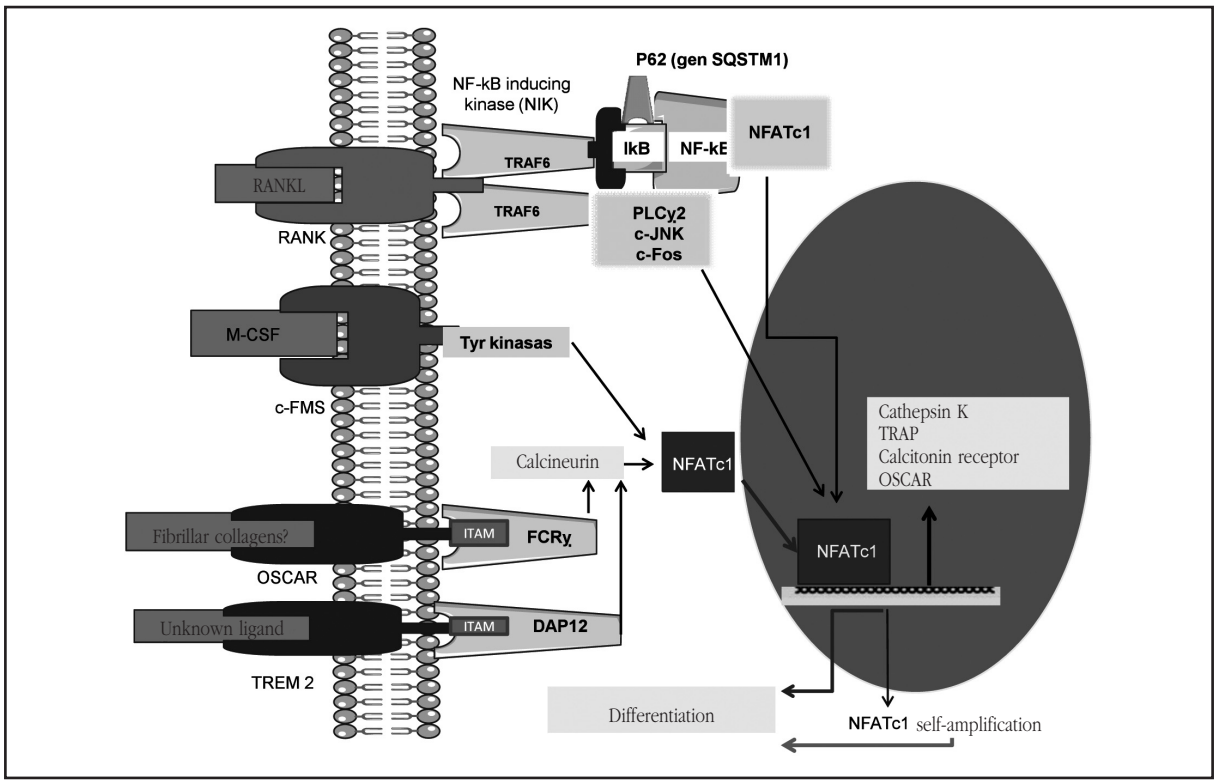
Two other members of the TNF superfamily have shown an osteoclastogenetic capability independent of RANKL. APRIL (a proliferation inducing ligand, TNFSF13) and BAFF (cell activating factor belonging to the TNF, also known as BLyS and TNFSF 13b), are capable, in *in vitro* culture, of inducing cells with the osteoclast phenotype from mononuclear precursors, although of a smaller size and with a lower number of nuclei and resorptive capacity than those induced by RANKL or LIGHT[71].

e) Origin of RANKL in osteoclast activation

Although the origin of RANKL which is involved in bone remodelling is classically thought to be the OBs, there have been a number of experimental findings which have cast doubt on this idea. In a pioneering study, Corral et al.[72] showed that the ablation of osteoblast progenitors by the administration of ganciclovir in mice bearing a thymidine-kinase transgene under the control of the osteocalcin promoter, did not cause any effects on the osteoclastic surfaces or on the markers for resorption, even after several weeks of follow up, in those in which the population of osteoblasts had disappeared from the bone surfaces. More recently, and using a similar transgenic murine model, Galli et al. observed that the absence of osteoblasts did not affect the levels at the baseline, or after being stimulated by PTH, of RANKL mRNA[73]. These studies indicate that the classic paradigm, which is that the RANKL which governs osteoclast activation comes from OBs or their precursors, should be revised[74].

OCs are formed in different locations in the skeleton with different purposes and with a variety of support cells charged with synthesising the RANKL necessary for their activation. For example, the femurs of mice which lack osteocytic RANKL develop a normal morphology, which indicates that the cortical modelling of the long bones is controlled by cells other than the osteocytes; whereas during chondral ossification, the main source of RANKL, which enables the reabsorptive action of the osteoclasts on the calcified

Figure 2. Canonic osteoclast activation and co-stimulatory signals. In addition to the canonic signals for proliferation and activation the osteoclast may receive other types of signals whose role could be highly important in inflammatory states



TRAF6: receptor associated factor TNF 6; PLC: phospholipase C; c-JNK: N-terminal kinase c-Jun; ITAM: immunoreceptor tyrosine-based activation motifs; DAP12: death associated protein 12; TREM 2: triggering receptor expressed on myeloid cells 2.

cartilage, are the hypertrophic chondrocytes[75]. The OC is also the effector cell for the erosion which characterises rheumatoid arthritis[76,77], and its activation is supported by the collaboration of the synovial cells of fibroblast lineage of the lymphocyte subclass Th17[78]. These facts suggest that the role of RANKL derived from the osteocytes could be limited to bone remodelling.

The osteocyte is a cell which provides a large amount of RANKL during physiological remodelling[79]. This fact is even more plausible from the biological point of view due to the known role of these cells in the detection of both mechanical and hormonal signals, which enables them to act at true regulators of bone remodelling, at least in physiological conditions. Using Cre-LoxP technology, which allows the modification of DNA in specific types of cells, Xiong et al.[74] caused the deletion of the osteocyte RANKL gene in mice and observed a reduction in OCs, with an increase in bone mass and of the markers for resorption, without alterations in the development of the skeleton or in dental eruption. In the laboratory of Takayanagi[79] the same results were obtained using similar technology. In summary, these studies demonstrate that osteocytes are the main producer cells for RANKL in physiological bone remodelling.

The RANKL which comes from the osteocyte is, therefore, the cytokine which controls physiological bone remodelling in response to mechanical and hormonal signals. The mechanism by which RANKL accesses OCs has not yet been sufficiently clarified. There is experimental evidence that the presence of soluble RANKL in the medium is sufficient to produce osteoclast expansion[80] and that the osteocytic projections express RANKL from the membrane and reach the bone surface where they make contact with the OCs and their precursors[64,81]. Finally, there is evidence that, both through the production of soluble RANKL and through that expressed in the membrane by the dendrites, the osteocytes control osteoclast activation. It has a dual role, since it also possesses the capability of producing sclerostin through the activation of its gene, SOST, and so contribute to the regulation of osteoformation[82].

Osteoclastic fusion

The osteoclast precursors are mononuclear cells which express TRAP, with no resorptive capability in *in vitro* cultures. The first step by which they acquire their functionality is through cell fusion, which then enables the formation of mature OCs. Understanding the intimate mechanisms which control this critical event in the physiopathology of remodelling is fundamental to the development of the new therapies.

In physiological conditions, the pre-OC TRAP cells + and the mature OCs are only found on the bone surfaces, which indicates that the fusion occurs in these locations. Using techniques of DNA subtraction in precursor cells stimulated by isolated M-CSF or M-CSF and RANKL, it was observed that DC-STAMP (dendritic cell-specific transmembrane protein) is an essential molecule for the fusion of mononuclear cells as a first step for the formation of active mature OCs. This transmembrane protein, discovered in 2000[83], is also expressed in dendritic cells and macrophages[84]. Its annulment in murine models provoked osteoporosis associated with a complete absence of fused mononuclear OCs as well as foreign-body giant cells. In these mice there persisted a moderate degree of resorptive activity in the mature cells, which indicates that their fundamental role is performed at fusion[85]. The regulation of DC-STAMP is complex and depends not only on the RANKL/RANK pathway but also on other independent factors, such as IL-32[86], Tal1 (T-cell acute lymphocytic leukemia 1)[87], LDLR (low-density lipoprotein receptor)[88], CCN2/CTGF (CCN family 2/connective tissue growth factor)[89] and vitamin E[90], among others, whose role is even less well known but which could be future targets of therapeutic interest.

OC fusion is promoted by other molecules such as the inflammatory cytokines. Among these, in addition to the actions already mentioned of RANKL, both TNF-α and LPS (lipopolysaccharide) are capable of inducing OC fusion under certain circumstances. For example, the action of TNF-α is specifically blocked by Ac anti-TNF-α, while the effect of LPS is partly blocked by these drugs, and completely blocked by polymyxin B[91]. The activation of these pathways is accompanied by intracellular signals dependent on kinases, and when inhibitors are used specific to these pathways OC fusion is reduced, while levels of DC-STAMP are not altered. These findings indicate that there are alternative pathways which regulate OC fusion independently of DC-STAMP, although it is not known if they exert physiological functions or only interfere with pathological processes[92].

Additional roles for osteoclasts

In addition to their function as the only cells capable of reabsorbing calcified bone matrix, OCs participate in other processes which we summarise below.

1. Stimulation of bone formation

Bone remodelling is a coupled process in which the osteoclast activity is followed by the action of the osteoblasts. The pharmacological inhibition of the former provokes a reduction in the latter, while the osteoforming stimulus is followed by a secondary increase in resorption. In principle, the model would appear to be simple, attributing to factors released from the matrix reabsorbed by the OCs a role in the recruitment of osteoblast[93,94]. However, in a study published in 2001, the Molecular Biology Group of the University of Hamburg demonstrated that, in some murine models of osteopetrosis and in a patient with the malignant infantile form, in spite of a functional alteration in the resorptive mechanism in the presence of a normal number of OCs, such as is produced with the annulment of the chloride channels ClC-7 C, there was normal bone formation[7]. This fact suggests that there are factors independent of the matrix reabsorbed by the OCs whose role in the coupling is probably more significant.

Among the mechanisms in which OCs intervene directly stimulating osteoformation, the following have been proposed[95]: on the one hand, ephrin B2, expressed in the osteoclast membrane, is capable of provoking an activation signal by bonding with its osteoblast receptor EphB4; also, sphingosine-1-phosphate is capable of causing the recruitment of osteoblast precursors to the remodelling sites[96], although treatment with analogues of this molecule has not shown significant results in the mending of fractures[97]. OC expresses, in addition, regulatory factors negative to osteoblasts, such as Atp6v0d2 (a subunit of the V-ATPase proton pump)[98]. Even though the physiological role of these molecular signals is not known, the findings which have been commented on suggest that the intervention of the OCs in remodelling is not limited to bone resorption, but that they also play a significant role in the coupling through molecular signals which participate in the recruitment, activation and inhibition of the osteoblasts.

2. Immune cells

Both OCs and OBs have the capability of responding to a wide variety of cytokines produced by the cells of the innate and adaptive immune systems[78,99-101]. The OCs contain all the mechanisms necessary for endocytosis and the processing of exogenous proteins coming from the material generated during resorption and in pathological situations such as osteomyelitis. In 2009, Kiesel et al.[102] demonstrated that the OCs could recruit T CD8+ FoxP3+ cells and present their antigens. These cells would play a regulatory role, whose function in non-inflammatory situations is unknown. A very attractive but non-proven hypothesis relates this capacity of the OCs as presenters of antigens to the existence of a large reservoir of CD8+ central memory T-lymphocytes in the bone marrow, the former participating in the latter's recruitment and maintenance[103].

The extraction of necrotic bone during a bacterial infection is another of the mechanisms in which OCs play a part in the immune response. In fact, in an elegant study in which murine models which emulated the biology of osteomyelitis and of periodontal implants were used, Li et al.[104] demonstrated that the functional inhibition of the OCs by bisphosphonates and by osteoprotogerin was associated with an increase in the quantity of necrotic cortical bone around the implant which acted as nests for the bacterial colonisation, while at the same time reducing the size of the drainage

orifice through which the opsonised bacteria were expelled to the exterior of the lesion. These data are highly significant since they suggest that the pharmacological inhibition of osteoclasts could be contraindicated in bone infections, as well as in the pathogenesis of osteonecrosis of the jaw, where bacterial colonisation is very important, and where OCs would play a key role, at least in its initial phases.

3. Articular cartilage

In those process in which the destruction of hyaline articular cartilage occurs, giant multinucleated cells have been observed which express the osteoclast phenotype (TRAP+, cathepsin K+, MMP9+, CD14−, HLA-DR−, CD45+, CD51+ and CD68+). These cells, called "chondroclasts" in some publications, have the capability of reabsorbing the cartilaginous matrix and have been implicated in the pathogeny of diseases such as rheumatoid arthritis or arthrosis[105]. Their specific role has not been established with any certainty, although there is various indirect evidence to suggest that they may play a significant role on articular damage. It is known that 30% of the total RANKL which is produced in arthritic joints is synthesised in the cartilage, essentially through the chondrocytes[106]. The soluble part of this cytokine acting like a paracrine, may participate, through osteoclast activation in locations of chondral-sinovial contact, in the pathology of erosion and of juxtaarticular osteopenia, which characterise rheumatoid lesions. Furthermore, even though it has not been demonstrated with sufficient certainty, chondrocytic RANKL may contribute to the transformation and activation of the mononuclear precursors, resulting in chondroclasts capable of degrading the cartilage. The mechanisms through which this action would occur is not yet known, but there is, undoubtedly, an interesting question to be asked based around about the possible therapeutic role of the inhibitors of RANKL in processes such as arthrosis.

4. Energy metabolism

Osteocalcin, a small peptide produced by osteoblasts, stimulates the secretion of insulin by the beta pancreatic cells, a finding of enormous importance in decisively implicating bone tissue in the hormonal control of energy metabolism[107]. This molecule has a number of the characteristics of a hormone: it is a specifically cellular product, synthesised in a pre-propeptide form and secreted into the circulation after a process of vitamin K-dependent gamma-carboxylation. This fact explains its great affinity for the bone matrix, which causes it to be released during bone resorption and converted into its active form after exposure to the acid pH of the resorption lacuna. In transgenic mice which lack V-ATPase activity, hypoinsulinemia and glucose intolerance associated with reduced levels of osteocalcin are observed[108]. A study which analysed the effects of alendronate in a small sample of patients showed reduced levels of infra-carboxylated osteocalcin which is associated inversely with an increase in body weight and of fat mass[109]. However, a review of the results of the FIT, HORIZON and FREEDOM studies did not show any alteration in these parameters, nor in glucose metabolism[110]. In summary, while animal models suggest a role for bone remodelling in the control of energy metabolism, the studies carried out in humans show discordant results which need to be clarified in the future[111].

Conclusions

The OC has been considered classically to be a cell whose function is exclusively that of bone remodelling, and which exhibits gregarious behaviour. However, in the last decade experimental findings have drastically transformed this over-simplistic view. The OC shares common origins with the cells of the immune system, both in the myeloid and the lymphoid series. Its role in articular inflammatory diseases such as rheumatoid arthritis is probably highly significant, since, to its well-known function as the only cell capable of dissolving the calcified bone matrix, are added new roles due to its capacity to secrete cytokines and as an antigen presenter cell. OCs, as extraordinarily dynamic cells, are therapeutic targets of enormous interest (Table 1) due to their participation in processes such as osteoporosis, arthrosis or cancer.

Bibliography

1. Seeman E. Modelling and remodelling. En: Bilezikian J, Raisz LG, Martin TJ, editores. Principles of bone biology (Third Edition). Filadelfia: Elsevier Inc; 2008;p.3-28.
2. Schett G. Biology, physiology and morphology of bone. En: Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR, editores. Kelley's Textbook of Rheumatology (Ninth Edition). Filadelfia: Saunders; 2013;p.61-6.
3. Goldring SR, Schett G. The role of the immune system in the bone loss of inflammatory artritis. En: Lorenzo J, Horowitz M, Choi Y, Schett G, Takayanagi H, editores. Osteoimmunology. Londres: Elsevier; 2011;p.301-22.
4. Olechnowicz SW, Edwards CM. Contributions of the host microenvironment to cancer-induced bone disease. Cancer Res 2014;74:1625-31.
5. Väänänen HK, Zhao H. Osteoclast function: biology and mechanisms En: Bilezikian JP, Raisz LG, Martin TJ. Principles of Bone Biology (Third Edition). Filadelfia: Elsevier Inc; 2008;p:193-209.
6. Graves AR, Curran PK, Smith C, Mindell JA. The Cl-/H+ antiporter ClC-7 is the primary chloride permeation pathway in lysosomes. Nature 2008;453:788-92.
7. Kornak U, Kasper D, Bösl MR, Kaiser E, Schweizer M, Schulz A, et al. Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell 2001;104:205-15.
8. Schaller S, Henriksen K, Sveigaard C, Heegaard AM, Hélix N, Stahlhut M, et al. The chloride channel inhibitor NS3736 prevents bone resorption in ovariectomized rats without changing bone formation. J Bone Miner Res 2004;19:1144-53.
9. Kasper D, Planells-Cases R, Fuhrmann JC, Scheel O, Zeitz O, Ruether K, et al. Loss of the chloride channel ClC-7 leads to lysosomal storage disease and neurodegeneration. EMBO J 2005;24:1079-91.
10. Kraft-Terry SD, Gendelman HE. Proteomic biosignatures for monocyte-macrophage differentiation. Cell Immunol 2011;271:239-55.
11. Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. Nat Rev Genet 2003;4:638-49.

Table 1. Summary of potential osteoclastic molecular targets

Molecular target	Characteristics	Consequences of pharmacological intervention	Citation
CX3CL1 (fractalkine)	Chemokine expressed in the osteoblast membrane with chemotactic and pro-adhesive action	Its blocking reduces the recruitment of osteoclast precursors	112
CX3CR1	CX3CL1 receptor expressed in the osteoclasts	Its blocking reduces the recruitment of osteoclast precursors	113
CXCL12/ CXCR4	Chemokine and its receptor both expressed in the osteoblasts	Its blocking reduces the arrival of OCs to the bone	114
S1P	Lipid mediator which controls the dynamics of the migration of the osteoclast precursors	The S1P agonists promote the arrival of the osteoblasts by means of the receptors S1PR1 and 2	29
CSF-1R (c-fms)	CSF receptor expressed in osteoclast precursors	Reduces osteoclast migration and activation in experimental arthritis	115
MAPK MK2	One of the most specific MAP kinases in the transduction of the intracellular osteoclastic signal	Inhibition of osteoclast activation without effecting osteoformation	116
NFATc1	Nuclear factor key to osteoclast activation	Inhibition of osteoclast activation	117
TGF-β	Multifunctional cytokine which regulates proliferation in different cell lines, very abundant in the bone	Blocking the TGF-β signal inhibits RANKL-induced osteoclastogenesis	118
Gα11 protein	Osteoblastic G protein involved in osteoclast activation	Its over-expression provokes osteopenia through a dual mechanism	119
PKC-δ	Central role in differentiation, fusion and function of OCs participating in the ERK signalling pathway of M-CSF and RANKL	Its inhibition alters the intracellular osteoclastic signal	120
DC-STAMP	Transmembrane protein which functions as an essential regulator of osteoclast fusion	Functional blocking of the mature osteoclasts	121

S1P: sphingosa-1-phosphate; CSF-1R: colony stimulator factor receptor 1; MAPK: mitogen-activated protein kinase; TGF-β: transforming growth factor beta; PKC-δ: protein kinase C delta; DC-STAMP: dendritic cell-specific transmembrane protein.

12. Xing L, Schwarz EM, Boyce BF. Osteoclast precursors, RANKL/RANK, and immunology. Immunol Rev 2005;208:19-29.
13. Kikuta J, Ishii M. Osteoclast migration, differentiation and function: novel therapeutic targets for rheumatic diseases. Rheumatology (Oxford) 2013;52:226-34.
14. Kotani M, Kikuta J, Klauschen F, Chino T, Kobayashi Y, Yasuda H, et al. Systemic circulation and bone recruitment of osteoclast precursors tracked by using fluorescent imaging techniques. J Immunol 2013;190:605-12.
15. Pang H, Wu XH, Fu SL, Luo F, Zhang ZH, Hou TY, et al. Co-culture with endothelial progenitor cells promotes survival, migration, and differentiation of osteoclast precursors. Biochem Biophys Res Commun 2013;430:729-34.
16. Mukherjee D, Zhao J. The role of chemokine receptor CXCR4 in breast cancer metastasis. Am J Cancer Res 2013;3:46-57.
17. Ziarek JJ, Liu Y, Smith E, Zhang G, Peterson FC, Chen J, et al. Fragment-based optimization of small molecule CXCL12 inhibitors for antagonizing the CXCL12/CXCR4 interaction. Curr Top Med Chem 2012;12:2727-40.
18. Han KH, Ryu JW, Lim KE, Lee SH, Kim Y, Hwang CS, et al. Vascular expression of the chemokine CX3CL1 promotes osteoclast recruitment and exacerbates bone resorption in an irradiated murine model. Bone 2014;61:91-101.
19. Karlström S, Nordvall G, Sohn D, Hettman A, Turek D, Ahlin K, et al. Substituted 7-Amino-5-thio-thiazolo[4,5-d]pyrimidines as potent and selective antagonists of the fractalkine receptor (CX3CR1). J Med Chem 2013;56:3177-90.
20. Kim CH, Wu W, Wysoczynski M, Abdel-Latif A, Sunkara M, Morris A, et al. Conditioning for hematopoietic transplantation activates the complement cascade and induces a proteolytic environment in bone marrow: a novel role for bioactive lipids and soluble

C5b-C9 as homing factors. Leukemia 2012;26:106-16.
21. Ratajczak MZ, Kim C, Janowska-Wieczorek A, Ratajczak J. The expanding family of bone marrow homing factors for hematopoietic stem cells: Stromal Derived Factor 1 Is not the only player in the game. Sci World J 2012; 2012:758512.
22. Gangoiti P, Arana L, Ouro A, Granado MH, Trueba M, Gómez-Muñoz A. Activation of mTOR and RhoA is a major mechanism by which Ceramide 1-phosphate stimulates macrophage proliferation. Cell Signal 2011;1:27-34.
23. Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, et al. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. Nature 2009;458:524-8.
24. Ishii M, Kikuta J, Shimazu Y, Meier-Schellersheim M, Germain RN. Chemorepulsion by blood S1P regulates osteoclast precursor mobilization and bone remodeling in vivo. J Exp Med 2010;207:2793-8.
25. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 2012;1:50-60.
26. Kikuta J, Kawamura S, Okiji F, Shirazaki M, Sakai S, Saito H, et al. Sphingosine-1-phosphate-mediated osteoclast precursor monocyte migration is a critical point of control in antibone-resorptive action of active vitamin D. Proc Natl Acad Sci USA 2013;110:7009-13.
27. Boyce BF. Sphingosine-1 phosphate: a new player in osteoimmunology. Dev Cell 2009;3:323-4.
28. Ishii M, Kikuta J. Sphingosine-1-phosphate signaling controlling osteoclasts and bone homeostasis. Biochim Biophys Acta 2013;1831:223-7.
29. Quint P, Ruan M, Pederson L, Kassem M, Westendorf JJ, Khosla S, et al. Sphingosine 1-phosphate (S1P) receptors 1 and 2 coordinately induce mesenchymal cell migration through S1P activation of complementary kinase pathways. J Biol Chem 2013;288:5398-406.
30. Takahashi N, Yamana H, Yoshiki S, Roodman GD, Mundy GR, Jones SJ, et al. Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures. Endocrinology 1988;122:1373-82.
31. Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, et al. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. J Exp Med 1999;190:1741-54.
32. Asagiri M, Takayanagi H. The molecular understanding of osteoclast differentiation. Bone 2007;40:251-64.
33. González Macías J, Olmos Martínez JM. Fisiopatología de la osteoporosis y mecanismo de acción de la PTH. Rev Osteoporos Metab Miner 2010;2 (Suppl 2);5-17.
34. Horowitz MC, Lorenzo JA. Immunologic regulation of bone development. Adv Exp Med Biol 2007;602:47-56.
35. Chai RC, Kouspou MM, Lang BJ, Nguyen CH, van der Kraan AG, Vieusseux JL, et al. Molecular stress inducing compounds increase osteoclast formation in a Heat Shock Factor 1 dependent manner. J Biol Chem 2014; Apr 1.
36. Asai K, Funaba M, Murakami M. Enhancement of RANKL-induced MITF-E expression and osteoclastogenesis by TGF-β. Cell Biochem Funct 2014; Feb 12. doi: 10.1002/cbf.3028.
37. Matsumoto T, Nagase Y, Iwasawa M, Yasui T, Masuda H, Kadono Y, et al. Distinguishing the proapoptotic and antiresorptive functions of risedronate in murine osteoclasts: role of the Akt pathway and the ERK/Bim axis. Arthritis Rheum 2011;12:3908-17.
38. Matsumoto T, Nagase Y, Hirose J, Tokuyama N, Yasui T, Kadono Y, et al. Regulation of bone resorption and sealing zone formation in osteoclasts occurs through protein kinase b-mediated microtubule stabilization. J Bone Miner Res 2013;5:1191-202.
39. Mao D, Epple H, Uthgenannt B, Novack DV, Faccio R. PLCgamma2 regulates osteoclastogenesis via its interaction with ITAM proteins and GAB2. J Clin Invest 2006;116:2869-79.
40. Hayden MS, Ghosh S. Shared principles in NF-kB signaling. Cell 2008;132:344-62.
41. Mantovani A. Molecular pathways linking inflammation and cancer. Curr Mol Med 2010;4:369-73.
42. Nakashima T, Hayashi M, Takayanagi H. New insights into osteoclastogenic signaling mechanisms. Trends Endocrinol Metab 2012;23:582-90.
43. Shaw JP, Utz PJ, Durand DB, Toole JJ, Emmel EA, Crabtree GR. Identification of a putative regulator of early T cell activation genes. Science 1998;241:202-5.
44. Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell 2002;6:889-901.
45. Asagiri M, Sato K, Usami T, Ochi S, Nishina H, Yoshida H, et al. Autoamplification of NFATc1 expression determines its essential role in bone homeostasis. J Exp Med 2005;202:1261-9.
46. Kuroda Y, Matsuo K. Molecular mechanisms of triggering, amplifying and targeting RANK signaling in osteoclasts. World J Orthop 2012;3:167-74.
47. Barrow AD, Raynal N, Andersen TL, Slatter DA, Bihan D, Pugh N, et al. OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12-deficient humans and mice. J Clin Invest 2011;121:3505-16.
48. Paradowska-Gorycka A, Jurkowska M. Structure, expression pattern and biological activity of molecular complex TREM-2/DAP12. Human Immunol 2013;74:730-7.
49. Nemeth K, Schoppet M, Al-Fakhri N, Helas S, Jessberger R, Hofbauer LC, et al. The role of osteoclast-associated receptor in osteoimmunology. J Immunol 2011;186:13-8.
50. Pelham CJ, Agrawal DK. Emerging roles for triggering receptor expressed on myeloid cells receptor family signaling in inflammatory diseases. Expert Rev Clin Immunol 2014;10:243-56.
51. Colonna M, Turnbull I, Klesney-Tait J. The enigmatic function of TREM-2 in osteoclastogenesis. Adv Exp Med Biol 2007;602:97-105.
52. Takahashi N, Maeda K, Ishihara A, Uehara S, Kobayashi Y. Regulatory mechanism of osteoclastogenesis by RANKL and Wnt signals. Front Biosci 2011;16:21-30.
53. Otero K, Shinohara M, Zhao H, Cella M, Gilfillan S, Colucci A, et al. TREM2 and β-catenin regulate bone homeostasis by controlling the rate of osteoclastogenesis. J Immunol 2012;188:2612-21.
54. Takayanagi H. The role of NFAT in osteoclast formation. Ann N Y Acad Sci 2007;1116:227-37.
55. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. Nat Rev Immunol 2014;14:36-49.
56. Meng S, Zhang L, Tang Y, Tu Q, Zheng L, Yu L, et al. BET inhibitor JQ1 blocks inflammation and bone destruction. J Dent Res 2014;93:657-62.
57. Yen ML, Hsu PN, Liao HJ, Lee BH, Tsai HF. TRAF-6 dependent signaling pathway is essential for TNF-related apoptosis-inducing ligand (TRAIL) induces osteoclast differentiation. PLoS One 2012;7:e38048.
58. Kitaura H, Kimura K, Ishida M, Sugisawa H, Kohara H, Yoshimatsu M, et al. Effect of cytokines on osteoclast formation and bone resorption during mechanical force loading of the periodontal membrane. Scientific World Journal 2014; Jan 19. doi:10.1155/2014/617032.
59. Iyer S, Margulies BS, Kerr WG. Role of SHIP1 in bone biology. Ann N Y Acad Sci 2013;1280:11-4.
60. Taniguchi R, Fukushima H, Osawa K, Maruyama T, Yasuda H, Weih F, et al. RelB-induced expression of Cot, a MAP3K family member, rescues RANKL-induced osteoclastogenesis in alymphoplasia mice by promoting NF-κB2 processing by IKKα. J Biol Chem 2014;289:7349-61.
61. Canalis E, Adams DJ, Boskey A, Parker K, Kranz L, Zanotti S. Notch signaling in osteocytes differentially regulates cancellous and cortical bone remodeling. J Biol Chem 2013;288:25614-25.
62. Smink JJ, Bégay V, Schoenmaker T, Sterneck E, de Vries TJ, Leutz A. Transcription factor C/EBPbeta isoform ratio regulates osteoclastogenesis through MafB. EMBO J 2009;28:1769-81.
63. Fu SL, Pang H, Xu JZ, Wu XH. C/EBPβ Mediates Osteoclast Recruitment by Regulating Endothelial Progenitor Cell Expression of SDF-1α. PLoS One 2014;9:e91217.
64. Zhao B, Takami M, Yamada A, Wang X, Koga T, Hu X, et al. Interferon regulatory factor-8 regulates bone metabolism by suppressing osteoclastogenesis. Nat

Med 2009;15:1066-71.
65. Park-Min KH, Lee EY, Moskowitz NK, Lim E, Lee SK, Lorenzo JA, et al. Negative regulation of osteoclast precursor differentiation by CD11b and β2 integrin-B-cell lymphoma 6 signaling. J Bone Miner Res 2013;28:135-49.
66. Kim N, Kadono Y, Takami M, Lee J, Lee SH, Okada F, et al. Osteoclast differentiation independent of the TRANCE–RANK–TRAF6 axis. J Exper Med 2005; 202:589-95.
67. Mellis DJ, Itzstein C, Helfrich MH, Crockett JC. The skeleton: a multi-functional complex organ. The role of key signalling pathways in osteoclast differentiation and in bone resorption. J Endocrinol 2011;211:131-43.
68. Ware CF. Targeting lymphocyte activation through the lymphotoxin and LIGHT pathways. Immunol Rev 2008;223:186-201.
69. Ware CF, Sedy J. TNF superfamily networks: bidirectional and interference pathways of the Herpesvirus Entry Mediator (TNFSF14). Curr Opin Immunol 2011;23:627-31.
70. Hemingway F, Kashima TG, Knowles HJ, Athanasou NA. Investigation of osteoclastogenic signalling of the RANKL substitute LIGHT. Exper Mol Pathol 2013;94:380-5.
71. Hemingway F, Taylor R, Knowles HJ, Athanasou NA. RANKL-independent human osteoclast formation with APRIL, BAFF, NGF, IGF I 2 and IGF II. Bone 2011;48:938-44.
72. Corral DA, Amling M, Priemel M, Loyer E, Fuchs S, Ducy P. Dissociation between bone resorption and bone formation in osteopenic transgenic mice. Proc Natl Acad Sci USA 1998;95:13835-40.
73. Galli C, Fu Q, Wang W, Olsen BR, Manolagas SC, Jilka RL, et al. Commitment to the osteoblast lineage is not required for RANKL gene expression. J Biol Chem 2009;284:12654-62.
74. Xiong J, O'Brien CA. Osteocyte RANKL: New Insights into the control of bone remodeling. J Bone Miner Res 2012;27:499-505.
75. Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol 1998;152:943-51.
76. Pettit AR, Ji H, von Stechow D, Müller R, Goldring SR, Choi Y, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001;159:1689-99.
77. Goldring SR, Purdue PE, Crotti TN, Shen Z, Flannery MR, Binder NB, et al. Bone remodelling in inflammatory arthritis. Ann Rheum Dis 2013;72:52-55.
78. Arboleya L, Castañeda S. Osteoimmunology. Reumatol Clin 2013;9:303-15.
79. Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med 2011;17:1231-4.
80. Zhao S, Kato Y, Zhang Y, Harris S, Ahuja SS, Bonewald LF. MLO-Y4 osteocyte-like cells support osteoclast formation and activation. J Bone Miner Res 2002;17:2068-79.
81. Kurata K, Heino TJ, Higaki H, Vaananen HK. Bone marrow cell differentiation induced by mechanically damaged osteocytes in 3D gel-embedded culture. J Bone Miner Res 2006;21:616-25.
82. Van Bezooijen RL, Roelen BAJ, Visser A, Wee-Pals L, de Wilt E, Karperien M, et al. Sclerostin is an osteocyte-expressed negative regulador of bone formation, but not a classical BMP antagonist. J Exp Med 2004;199:805-14.
83. Hartgers FC, Vissers JL, Looman MW, van Zoelen C, Huffine C, Figdor CG, et al. DC-STAMP, a novel multimembrane-spanning molecule preferentially expressed by dendritic cells. Eur J Immunol 2000;30:3585-90.
84. Xing L, Xiu Y, Boyce BF. Osteoclast fusion and regulation by RANKL-dependent and independent factors. World J Orthop 2012;3:212-22.
85. Yagi M, Ninomiya K, Fujita N, Suzuki T, Iwasaki R, Morita K, et al. Induction of DC-STAMP by alternative activation and downstream signaling mechanisms. J Bone Miner Res 2007;22:992-1001.
86. Kim YG, So MW, Koo BS, Chang EJ, Song SJ, Lee CK, et al. The influence of interleukin-32γ on osteoclastogenesis with a focus on fusion-related genes. J Clin Immunol 2012;32:201-6.
87. Courtial N, Smink JJ, Kuvardina ON, Leutz A, Göthert JR, Lausen J. Tal1 regulates osteoclast differentiation through suppression of the master regulator of cell fusion DC-STAMP. FASEB J 2012;26:523-32.
88. Okayasu M, Nakayachi M, Hayashida C, Ito J, Kaneda T, Masuhara M, et al. Low-density lipoprotein receptor deficiency causes impaired osteoclastogenesis and increased bone mass in mice because of defect in osteoclastic cell-cell fusion. J Biol Chem 2012;287:19229-41.
89. Nishida T, Emura K, Kubota S, Lyons KM, Takigawa M. CCN family 2/connective tissue growth factor (CCN2/CTGF) promotes osteoclastogenesis via induction of and interaction with dendritic cell-specific transmembrane protein (DC-STAMP). J Bone Miner Res 2011;26:351-63.
90. Fujita K, Iwasaki M, Ochi H, Fukuda T, Ma C, Miyamoto T, et al. Vitamin E decreases bone mass by stimulating osteoclast fusion. Nat Med 2012;18:589-94.
91. Hotokezaka H, Sakai E, Ohara N, Hotokezaka Y, Gonzales C, Matsuo K, et al. Molecular analysis of RANKL-independent cell fusion of osteoclast-like cells induced by TNF-alpha, lipopolysaccharide, or peptidoglycan. J Cell Biochem 2007;101:122-34.
92. Zhu M, Van Dyke TE, Gyurko R. Resolvin E1 regulates osteoclast fusion via DC-STAMP and NFATc1. FASEB J 2013;27:3344-53.
93. Bonewald LF, Mundy GR. Role of transforming growth factor-beta in bone remodeling. Clin Orthop Relat Res 1990;250:261-76.
94. Mohan S, Baylink DJ. Insulin-like growth factor system components and the coupling of bone formation to resorption. Horm Res 1996;45(Suppl 1):59-62.
95. Tamma R, Zallone A. Osteoblast and osteoclast crosstalks: from OAF to Ephrin. Inflamm Allergy Drug Targets 2012;11:196-200.
96. Boyce BF. Advances in osteoclast biology reveal potential new drug targets and new roles for osteoclasts. J Bone Miner Res 2013;28:711-22.
97. Heilmann A, Schinke T, Bindl R, Wehner T, Rapp A, Haffner-Luntzer M, et al. Systemic treatment with the sphingosine-1-phosphate analog FTY720 does not improve fracture healing in mice. J Orthop Res 2013 Jul 1.doi: 10.1002/jor.22426.
98. Lee SH, Rho J, Jeong D, Sul JY, Kim T, Kim N, et al. v-ATPase V0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. Nat Med 2006;12:1403-9.
99. Jones D, Glimcher LH, Aliprantis AO. Osteoimmunology at the nexus of arthritis, osteoporosis, cancer, and infection. J Clin Invest 2011;121:2534-42.
100. Manilay JO, Zouali M. Tight relationships between B lymphocytes and the skeletal system. Trends Mol Med 2014;Apr 10.doi: 10.1016/j.molmed.2014.03.003.
101. Feng W, Xia W, Ye Q, Wu W. Osteoclastogenesis and osteoimmunology. Front Biosci 2014;19:758-6.
102. Kiesel JR, Buchwald ZS, Aurora R. Cross-presentation by osteoclasts induces FoxP3 in CD8+ T cells. J Immunol 2009;182:5477-87.
103. Mazo IB, Honczarenko M, Leung H, Cavanagh LL, Bonasio R, Weninger W. Bone marrow is a major reservoir and site of recruitment for central memory CD8+ T cells. Immunity 2005;22:259-70.
104. Li D, Gromov K, Proulx ST, Xie C, Li J, Crane DP, et al. Effects of antiresorptive agents on osteomyelitis: novel insights into the pathogenesis of osteonecrosis of the jaw. Ann N Y Acad Sci 2010;1192:84-94.
105. Knowles HJ, Moskovsky L, Thompson MS, Grunhen J, Cheng X, Kashima TG, et al. Chondroclasts are mature osteoclasts which are capable of cartilage matrix resorption. Virchows Arch 2012;461:205-10.
106. Martínez-Calatrava MJ, Prieto-Potín I, Roman-Blas JA, Tardio L, Largo R, Herrero-Beaumont G. RANKL synthesized by articular chondrocytes contributes to juxta-articular bone loss in chronic artritis. Arthritis Res Ther 2012;14:R149.
107. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;130:456-69.
108. Ferron M, Wei J, Yoshizawa T, Del Fattore A, De Pinho RA,

Teti A, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell 2010;142:296-308.
109. Schafer AL, Sellmeyer DE, Schwartz AV, Rosen CJ, Vittinghoff E, Palermo L, et al. Change in undercarboxylated osteocalcin is associated with changes in body weight, fat mass, and adiponectin: parathyroid hormone (1-84) or alendronate therapy in postmenopausal women with osteoporosis (the PaTH study). J Clin Endocrinol Metab 2011;96:1982-9.
110. Schwartz AV, Schafer AL, Grey A, Vittinghoff E, Palermo L, Lui LY, et al. Effects of antiresorptive therapies on glucose metabolism: results from the FIT, HORIZON-PFT, and FREEDOM trials. J Bone Miner Res 2013;28:1348-54.
111. Karsenty G, Ferron M. The contribution of bone to whole-organism physiology. Nature 2012;481:314-20.
112. Koizumi K, Saitoh Y, Minami T, Takeno N, Tsuneyama K, Miyahara T, et al. Role of CX3CL1/fractalkine in osteoclast differentiation and bone resorption. J Immunol 2009;183:7825-31.
113. Hoshino A, Ueha S, Hanada S, Imai T, Ito M, Yamamoto K, et al. Roles of chemokine receptor CX3CR1 in maintaining murine bone homeostasis through the regulation of both osteoblasts and osteoclasts. J Cell Sci 2013;126:1032-45.
114. Shahnazari M, Chu V, Wronski TJ, Nissenson RA, Halloran BP. CXCL12/CXCR4 signaling in the osteoblast regulates the mesenchymal stem cell and osteoclast lineage populations. FASEB J 2013;27:3505-13.
115. Toh ML, Bonnefoy JY, Accart N, Cochin S, Pohle S, Haegel H, et al. A CSF-1 Receptor monoclonal antibody has potent bone and cartilage protective effects in experimental arthritis. Arthritis Rheumatol 2014;Mar 12. doi: 10.1002/art.38624.
116. Braun T, Lepper J, Ruiz Heiland G, Hofstetter W, Siegrist M, Lezuo P, et al. Mitogen-activated protein kinase 2 regulates physiological and pathological bone turnover. J Bone Miner Res 2013;28:936-47.
117. Intini G, Katsuragi Y, Kirkwood KL, Yang S. Alveolar bone loss: mechanisms, potential therapeutic targets, and interventions. Adv Dent Res 2014;26:38-46.
118. Yasui T, Kadono Y, Nakamura M, et al. Regulation of RANKL-induced osteoclastogenesis by TGF-beta through molecular interaction between Smad3 and Traf6. J Bone Miner Res 2011;26:1447-56.
119. De la Cruz A, Mattocks M, Sugamori KS, Grynpas MD, Mitchell J. Reduced trabecular bone mass and strength in mice overexpressing Gα11 protein in cells of the osteoblast lineage. Bone 2014;59:211-22.
120. Khor EC, Abel T, Tickner J, Chim SM, Wang C, Cheng T,et al. Loss of protein kinase C-δ protects against LPS-induced osteolysis owing to an intrinsic defect in osteoclastic bone resorption. PLoS One 2013;8:e70815.
121. Zhang C, Dou C, Xu J, Dong S. DC-STAMP, the key fusion-mediating molecule in osteoclastogenesis. J Cell Physiol 2014;doi: 10.1002/jcp.24553.