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Calcium Signaling in Osteoclasts

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Abstract

It has long been known that many bone diseases, including osteoporosis, involve abnormalities in osteoclastic bone resorption. As a result, there has been intense study of the mechanisms that regulate both the differentiation and bone resorbing function of osteoclast cells. Calcium (Ca^{2+}) signaling appears to play a critical role in the differentiation and functions of osteoclasts. Cytoplasmic Ca^{2+} oscillations occur during RANKL-mediated osteoclastogenesis. Ca^{2+} oscillations provide a digital Ca^{2+} signal that induces osteoclasts to up-regulate and autoamplify nuclear factor of activated T cells c1 (NFATc1), a Ca^{2+}/calcineurin-dependent master regulator of osteoclastogenesis. Here we review previous studies on Ca^{2+} signaling in osteoclasts as well as recent breakthroughs in understanding the basis of RANKL-induced Ca^{2+} oscillations, and we discuss possible molecular players in this specialized Ca^{2+} response that appears pivotal for normal bone function.

Introduction

Bones are dynamic living organs that are constantly renewed throughout one's life. This constant and balanced bone turnover relies on the process of bone remodeling mediated by osteoblasts that form bone and osteoclasts that resorb bone [57]. Imbalance between osteoblastic bone production and osteoclastic bone resorption favoring bone resorption is known to occur in many bone diseases such as postmenopausal osteoporosis, arthritis, and bone tumors [36,43]. Accordingly, most drugs used in the treatment of osteoporosis are anit-resorptive in nature. Bisphosphonates, estrogen, and calcitonin are currently the main pharmacological approaches for prevention of bone loss [28,35]. However, there are many side effects from the long-term use of these drugs such as constipation, diarrhea, tumorigenic and cardiovascular effects, and osteonecrosis of the jaw [28,35]. As a result, there have been considerable efforts to develop new therapeutic targets for the treatment or prevention of bone loss.

The activation of the receptor activator of nuclear factor-κB (RANK) by its specific ligand (RANKL) is an essential initiating signal for osteoclastogenesis, One of the key downstream signals in the RANK/RANKL pathway is the Ca^{2+} dependent calcineurin/NFAT pathway, implicating a significant role for Ca^{2+} signaling. We will discuss the RANKL-dependent pathway and the role of Ca^{2+} signaling in more detail below.

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Ca^{2+} mobilization in osteoclasts

Ca^{2+} serves as a ubiquitous second messenger that can specifically mediate and regulate a variety of downstream signaling pathways [1]. Many different stimuli have been shown to regulate Ca^{2+} concentrations in osteoclasts. Extracellular acidification caused a decrease in intracellular Ca^{2+} concentration in isolated chicken osteoclasts which in turn enhanced attachment of cells to bone matrix [45]. Yu and Ferrier [56] reported that ATP triggers a transient rise in intracellular Ca^{2+} concentrations in rabbit osteoclasts. They concluded that P_2 purinergic receptors are involved in this rise of Ca^{2+}. The same group demonstrated that the ATP-induced Ca^{2+} rise was smaller and more transient in Ca^{2+} free buffer, suggesting that activation of Ca^{2+} influx contributes to the Ca^{2+} signal in osteoclasts. $\alpha v\beta_3$ integrin receptors are highly expressed in osteoclasts and known to be important for the function and adhesion of osteoclasts on the bone matrix [8,10,15,16]. Activation of the integrin receptor by specific peptides caused a transient Ca^{2+} response in the absence of extracellular Ca^{2+} [38]. Xia and Ferrier [50] reported that mechanical perturbation of osteoclasts induced a Ca^{2+} mobilization response whose amplitude and duration were dependent on the extracellular Ca^{2+} concentration. Radding et al. [34] observed intracellular Ca^{2+} puffs in acid-secreting osteoclasts, which they suggest may be involved in signaling acid secretion for bone resorption.

The most common mechanism of Ca^{2+} mobilization by extracellular stimuli involves activation of phospholipase C (PLC)-coupled receptors, leading to the production of inositol-1,4,5- trisphosphate (IP_3). IP_3 binds to and activates the IP_3 receptor (IP_3R) resulting in Ca^{2+} release from the endoplasmic reticulum (ER) [1]. IP_3 has been shown to induce Ca^{2+} release from bone cells including osteoblasts and osteoclasts [11]. Yaroslavskiy et al. [52] demonstrated that the IP_3R type 1 is required for activation of Ca^{2+}-dependent μ-calpain and nitric oxide-induced Ca^{2+} mobilization in osteoclasts. Morikawa et al. [31], using RT-PCR and immunofluorescence, reported the presence in rat osteoclasts of all three isotypes of IP_3R. Interestingly, Morikawa et al. found that IP_3R type 3 is localized to podosomes where osteoclasts adhere to bone, suggesting a potential role of IP_3R in the formation or function of podosomes. Malgaroli et al. [24] reported that osteoclast cells sense high extracellular Ca^{2+} and respond with increased intracellular Ca^{2+} transients that may be linked to activation of PLC. Similarly, Zaidi et al. [58] reported that high extracellular Ca^{2+} induces elevation of intracellular Ca^{2+} in isolated rat osteoclasts. They also suggest that extracellular Ca^{2+} regulates bone resorption activity of osteoclasts. Subsequently it was confirmed by Seuwen et al. [37] that high extracellular Ca^{2+} elicits Ca^{2+} release associated with production of inositol phosphate in osteoclast-like cells, suggesting the involvement of Ca^{2+} sensing receptors in Ca^{2+} signaling in osteoclasts. By use of a Ca^{2+} receptor knockout mouse as well as a Ca^{2+} receptor dominant negative construct, Mentaverri et al. [27] provided evidence that Ca^{2+} sensing receptors play a critical role in osteoclast differentiation and apoptosis.

Involvement of the ryanodine receptor (RyR), an intracellular Ca^{2+} release channel, in the activation of Ca^{2+} mobilization in osteoclasts was investigated by several groups. Zaidi et al. [60] reported that Ni^{2+} induced cytosolic Ca^{2+} release in rat osteoclasts and this response was blocked by ryanodine, suggesting the presence of Ca^{2+} releasing ryanodine receptors. Similarly, Shankar [40] showed that low concentrations of caffeine, a RyR agonist, induce cytosolic Ca^{2+} mobilization in isolated rat osteoclasts. One group has suggested that RyRs are expressed in the plasma membrane of osteoclasts, functioning as extracellular Ca^{2+} sensing receptor [30,61]. However, this idea has not as yet gained general acceptance [6].

Thus, various stimuli activated Ca^{2+} signaling in osteoclasts, and the signal appears to be comprised of Ca^{2+} released from intracellular stores, and also entering the cell across the

plasma membrane. One major mechanism for activating Ca^{2+} entry across the plasma membrane is the store-operated pathway [33]. To determine the role played by store-operated channels in osteoclasts, Zaidi et al. [59] examined the effects of thapsigargin, a membrane permeant inhibitor of the ER Ca^{2+} transporting ATPase. When applied to osteoclasts, thapsigargin increased intracellular Ca^{2+} concentration in a Ca^{2+} free buffer, indicating discharge of Ca^{2+} from internal stores. . The thapsigargin-induced Ca^{2+} elevation was augmented upon restoration of extracellular Ca^{2+}, indicating a stimulated Ca^{2+} influx in osteoclasts. Activation of Ca^{2+} influx by thapsigargin is generally taken as evidence for the store-operated Ca^{2+} entry pathway [3]. This type of Ca^{2+} entry is blocked by several pharmacological agents such as 2-aminoethyldiphenyl borate, SKF-96365, and low concentration (1–5 μM) of Gadolinium (Gd^{3+}), and as discussed below, these agents have been shown to affect osteoclast signaling function [18,26]. Consistent with Zaidi's observation, Shankar et al. [39] showed that store-depletion by the Ca^{2+} ionophore, ionomycin, elicits a rapid and transient Ca^{2+} mobilization in Ca^{2+} free media, which becomes sustained after restoration of extracellular Ca^{2+}, indicating that two phase of Ca^{2+} signaling occurs in osteoclasts, Ca^{2+} release followed by activation of Ca^{2+} influx upon store depletion.

RANKL-evoked Ca^{2+} mobilization in osteoclasts

Two hematopoietic factors, receptor activator of nuclear factor-κB ligand (RANKL) and macrophage-colony stimulating factor, are essential for osteoclastogenesis, [5,17,21,23,53,55]. Mature and functional osteoclasts are formed from bone marrow-derived monocyte/macrophage precursor cells in the presence of these two required factors. Importantly, Takayanagi [44] reported that cytosolic Ca^{2+} oscillations occur not in response to IL-1 but rather to RANKL during osteoclastogenesis, suggesting the presence of signaling pathways specifically activated by RANKL. These same authors reported that, on the basis of genome wide screening, NFATc1 is specifically up-regulated by RANKL. Interestingly, NFATc1 is known to be regulated by Ca^{2+}/calmodulin-dependent calcineurin. They proposed that Ca^{2+} oscillations provide a prolonged digital Ca^{2+} signal which activates calcineurin, leading to up-regulation (and autoamplification) of NFATc1 and thereby promotes osteoclastogenesis. In support of this idea, transient Ca^{2+} mobilization by Ca^{2+} ionophores such as ionomycin failed to up-regulate NFATc1 [44]. Furthermore, they showed that ectopic over-expression of NFATc1 is sufficient to induce osteoclastogenesis even in the absence of RANKL. Subsequently, many other groups confirmed the importance of RANKL-induced Ca^{2+} oscillations in osteoclastogenesis. Yang and Li [51] showed that genetic ablation of regulator of G-protein signaling 10 (RGS10) abolishes RANKL-induced Ca^{2+} oscillations, leading to impaired up-regulation of NFATc1 and osteoclastogenesis. These authors demonstrated that over-expression of NFATc1 partially rescues the impaired osteoclastogenesis in $RGS10^{-/-}$ in the absence of RANKL. By using a proteomic technique, Yoon et al. [54] found that Lyn, a Src family tyrosine kinase, was down-regulated in RANKL-induced osteoclastogenesis which might suggest a role of Lyn as a negative regulator during osteoclast differentiation. Consistent with this idea, the same group observed that knockdown of Lyn resulted in an increase in NFATc1 expression accompanying Ca^{2+} oscillations. Knockdown of Lyn also promoted the formation of both TRAP-positive multinucleated osteoclasts and resorption pits [54]. In addition, several studies have indicated that RANKL induces a more immediate and transient Ca^{2+} elevation in isolated, mature osteoclasts. Komarova et al. [20] observed that RANKL triggers an intracellular Ca^{2+} rise in isolated rat osteoclasts. The Ca^{2+} rise was apparently derived solely from an intracellular Ca^{2+} source, and signaled translocation of NFκB and enhanced osteoclast survival. Chamoux et al. [7] reported that RANKL elicited a rapid and sustained intracellular Ca^{2+} rise in osteoclasts cultured from human blood. In this case, extracellular Ca^{2+} influx appeared to be the major source of the Ca^{2+} signal.

Role of Ca^{2+} influx in RANKL-evoked Ca^{2+} signaling

As described earlier, cytosolic Ca^{2+} oscillations occur during RANKL-mediated osteoclastogenesis [44]. These oscillations are initiated by activation of co-stimulatory receptors such as the osteoclast-associated receptor and TREM2 after binding of RANKL to RANK [19]. Subsequently, these receptors recruit the spleen tyrosine kinase, which activates PLCγ by phosphorylation in a concert with Tec-family kinases [41,42]. Activation of PLCγ triggers the production of IP_3, resulting in release of Ca^{2+} from the ER. It is noteworthy that in most cell types, activation of receptors coupled to PLC by high concentrations of agonists triggers Ca^{2+} release from the ER followed by Ca^{2+} influx through store-operated Ca^{2+} entry (SOCE) [1,33]. Lower and more physiological concentrations of receptor agonists induce repetitive Ca^{2+} oscillations [2,46], similar to those seen in RANKL-mediated osteoclast differentiation. SOCE is necessary to refill the store in order to maintain Ca^{2+} oscillations, which run down in the absence of SOCE [4]. Alternatively, SOCE in some instances may directly provide activator Ca^{2+} to trigger downstream responses [9]. In either case, store-operated Ca^{2+} (SOC) channel would be expected to play an important physiological role in RANKL-induced Ca^{2+} signaling. Accordingly, Mentaverri et al. [26] reported that inhibition of SOCE by two relatively non-specific SOC channel blockers, 2-aminoethyldiphenyl borate and SKF-96365, diminished bone resorption activity of osteoclasts. Furthermore, a low concentration of Gd^{3+}, which is a relatively specific SOC blocker, abolished RANKL-induced Ca^{2+} oscillations [18]. The same group also demonstrated that knockdown of STIM1 which is a recently identified SOC protein, significantly reduces the frequency of RANKL-induced Ca^{2+} oscillations, suggesting that SOCE is an important component of the Ca^{2+} oscillations/calcineurin/NFATc1-dependent signaling complex induced by RANKL.

However, the molecular identity of the channels responsible for the Ca^{2+} influx is far from a settled issue. Several recent studies have focused on transient receptor potential (TRP) channels as candidates for the channels underlying Ca^{2+} influx in RANKL-induced Ca^{2+} oscillations. The vanilloid TRP5 (TRPV5) channels are apparently expressed in human and murine bone samples and in cultured osteoclasts [47]. The TRPV5 was localized to the ruffled border membrane of osteoclasts. Using a mouse model lacking the TRPV5 gene, Van der Eerden et al. [47] concluded that TRPV5 plays a critical role in the function of osteoclasts since in vitro resorption activity was attenuated in $TRPV5^{-/-}$ mice. However, the $TRPV5^{-/-}$ mice actually displayed enhanced osteoclastogenesis [47]. Nonetheless, Hoenderop et al. [14] observed that mice lacking TRPV5 exhibited a decrease in trabecular and cortical thickness of long bones. Furthermore, Masuyama et al. [25] reported an increase in bone mass in $TRPV4^{-/-}$ mice which they attributed to impaired resorption activity of osteoclasts. The mRNA levels of NFATc1 were attenuated in cultured osteoclasts derived from $TRPV4^{-/-}$ mice, while osteoblast phenotypes were not affected, suggesting TRPV4 solely contributes to the differentiation and function of osteoclasts. However, the same group found that TRPV4 was not necessary to generate or to maintain Ca^{2+} oscillations in osteoclasts since there was no significant difference in the characteristic of Ca^{2+} oscillations between WT and $TRPV4^{-/-}$ mice (percentage of oscillating cells, frequency and amplitude of Ca^{2+} oscillations). They concluded that TRPV4 is more likely involved in Ca^{2+} influx in large and mature osteoclasts after Ca^{2+} oscillations have disappeared. They used 4α-PDD, a specific TRPV4 agonist to show that TRPV4-mediated Ca^{2+} response peaks at the later stage of osteoclasts, suggesting there might be another channel underlying Ca^{2+} influx at the early stages of osteoclast differentiation. Chamoux et al. [7] showed that knockdown of TRPV5 using TRPV5-targeted siRNA leads to inhibition of the RANKL-induced Ca^{2+} influx in human osteoclasts, which they suggest that TRPV5 is a major player responsible for the RANKL-induced intracellular Ca^{2+} rise in human osteoclasts. Despite the apparent role of TRPV5 in RANKL-induced Ca^{2+} signaling in osteoclasts, knockdown of TRPV5

actually promoted bone resorption. On this basis, Chamoux et al. [7] have suggested that TRPV5 may function as a negative regulator of bone homeostasis, similar to the inhibitory role of Lyn on the resorptive activity of osteoclasts [54]. In addition to the Ca^{2+} channels described above, Moonga et al., clearly demonstrated the expression of a Na^{+}/Ca^{2+} exchanger that contributes to the functional bone resorbing activity of isolated rat osteoclasts [29].

Concluding remarks

Changes in intracellular Ca^{2+} concentrations are known to function as universal triggers of diverse signaling pathways, including enzyme activation, cell survival and differentiation. Accordingly, alterations in intracellular Ca^{2+} concentrations by different stimuli also appear to regulate differentiation and functions of osteoclasts. A summary of the variety of stimuli that can affect Ca^{2+} signaling in osteoclasts is given in Table 1. There has been much progress in understanding the molecular basis for differentiation and activation of osteoclasts in the last decade following the discovery of RANKL [21,23,53]. Yet, many questions still remain, especially regarding the function of Ca^{2+} signaling. For example, bone mass was increased in TRPV4$^{-/-}$ mice despite the fact that there was no effect on RANKL-induced Ca^{2+} oscillations in osteoclasts [25]. Interestingly, Kuroda et al. [22] reported that RANKL-induced Ca^{2+} oscillations are lost in osteoclasts from IP_3R type 2$^{-/-}$ mice, resulting in abolished osteoclastogenesis. However, the osteoclastogenesis returned in the absence of Ca^{2+} oscillations when the osteoclasts from IP_3R type 2$^{-/-}$ mice were co-cultured with osteoblasts. Furthermore, when osteoclastogenesis was induced in IP_3R type 2$^{-/-}$ cells lacking Ca^{2+} oscillations, i.e., when mediated by co-culture with osteoblasts, activation and translocation of NFATc1 were still induced, albeit partially. This oscillation-independent induction of NFATc1 was observed even in the presence of FK506, a calcineurin inhibitor. Collectively, these findings suggest the existence of a possible alternative pathway that is Ca^{2+} oscillations/calcineurin-independent and activated by interaction of osteoblasts and osteoclasts.

An obvious take-home message from much of the above discussion is the degree of uncertainty with regard to the Ca^{2+} signaling mechanisms involved in osteoclastogenesis, especially with regard to the route of Ca^{2+} entry across the plasma membrane. Although there has been limited work in this area, there is ample evidence for the SOCE pathway in osteoclasts [26]. In other cells of the hematopoietic lineage SOCE is known to play an essential role in activating NFAT signaling [12,32]. In just the past few years, the molecular components underlying SOCE have been revealed, in particular the components of the SOCE channels, Orai1, 2 and 3 [13,49,62]. Genetic deletion of Orai1 in mice abolishes SOCE in some, but not all, hematopoietic cells [48]. Zhou et al.[63] recently reported that knockdown of Orai1 abrogates the osteoclastogenesis of human monocytes by suppressing multinucleation of precursor cells, suggesting the involvement of Orai1 channels in osteoclastogenesis. However, many questions still remain yet. What is the mechanism by which Orai1 regulates osteoclastogenesis? It is possible that Ca^{2+} influx through Orai1 channels activates NFAT, but this has not been demonstrated. How does Orai1 influence the cell-cell fusion of the osteoclasts? Is either Orai2 or 3 also involved? Is Orai1 needed for RANKL-induced Ca^{2+} oscillations? And what role do the store-operated Orai channels play in the process of bone resorption?

Further progress in understanding the significance of SOCE and Orai channels in Ca^{2+} oscillations/calcinerin/NFATc1-dependent osteoclastogenesis may provide a more complete molecular picture of the mechanisms underlying Ca^{2+} signaling in bone. It will help our insight in developing new therapeutic approaches for treatment of many bone diseases in which excessive osteoclastic resorption is involved.

Research Highlights

Calcium signaling plays a significant role in the process of osteoclastogenesis.

The RANKL receptor utilizes calcium signaling and activation of NFAT to drive differentiation of osteoclasts.

Recent studies demonstrate that a key component of osteoclast calcium signaling involves influx through plasma membrane channels, including members of the TRP channel superfamily, as well as store-operated channels.

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Table 1

Summary of Documented Calcium Signaling in Osteoclasts.

	Stimulus	Type of Ca^{2+} response	Significance of Ca^{2+} response	References
Isolated chicken osteoclast	Acidification	Decrease in $[Ca^{2+}]_i$	Promote podosome formation	[45]
Isolated rabbit osteoclast	ATP (50 μM)	Rapid and transient increase in $[Ca^{2+}]_i$	ND	[56]
Isolated rat osteoclast	Peptides that bind integrin	Rapid and transient increase in $[Ca^{2+}]_i$ around nucleus	ND	[38]
Isolated rabbit osteoclast	Mechanical perturbation	Rapid and transient increase in $[Ca^{2+}]_i$	ND	[50]
Bone-attached chicken osteoclast	Acid secretion	$[Ca^{2+}]_i$ puffs	Osteoclastic acid secretion	[34]
Human osteosarcoma lines, isolated rat osteoblastic and osteoclastic cells	IP_3	Transient increase in $[Ca^{2+}]_i$	ND	[11]
Isolated rat and chicken osteoclast	High extracellular Ca^{2+}	Rapid, various types of increase in $[Ca^{2+}]_i$	Osteoclast retraction	[24]
Isolated rat osteoclast	High extracellular Ca^{2+}	Rapid and sustained increase in $[Ca^{2+}]_i$	Resorption activity	[58]
Osteoclast-like cells GCT23	High extracellular Ca^{2+}	Rapid increase in $[Ca^{2+}]_i$	ND	[37]
Isolated rat osteoclast	Caffeine	Rapid and transient increase in $[Ca^{2+}]_i$	ND	[40]
RANKL-differentiated osteoclast	RANKL	Delayed and spontaneous $[Ca^{2+}]_i$ oscillations	Osteoclast differentiation	[44]
RANKL-differentiated osteoclast	RANKL	Delayed and spontaneous $[Ca^{2+}]_i$ oscillations	Osteoclast differentiation	[51]
RANKL-differentiated osteoclast	RANKL	Delayed and spontaneous $[Ca^{2+}]_i$ oscillations	Osteoclast differentiation	[54]
Isolated rat osteoclast	RANKL	Rapid and transient increase in $[Ca^{2+}]_i$	Promotion of cell survival by nuclear translocation of NFκB	[20]
Human osteoclast	RANKL	Rapid and sustained increase in $[Ca^{2+}]_i$	Resorption activity	[7]

ND: not determined

RANKL: receptor activator of nuclear factor-κB ligand