The Role of Ca\textsuperscript{2+}-NFATc1 Signaling and Its Modulation on Osteoclastogenesis

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Abstract: The increasing of intracellular calcium concentration is a fundamental process for mediating osteoclastogenesis, which is involved in osteoclastic bone resorption. Cytosolic calcium binds to calmodulin and subsequently activates calcineurin, leading to NFATc1 activation, a master transcription factor required for osteoclast differentiation. Targeting the various activation processes in osteoclastogenesis provides various therapeutic strategies for bone loss. Diverse compounds that modulate calcium signaling have been applied to regulate osteoclast differentiation and, subsequently, attenuate bone loss. Thus, in this review, we summarized the modulation of the NFATc1 pathway through various compounds that regulate calcium signaling and the calcium influx machinery. Furthermore, we addressed the involvement of transient receptor potential channels in osteoclastogenesis.

Keywords: osteoclast; calcium signaling; NFAT; transient receptor potential channels

1. Osteoclastogenesis in Bone Remodeling

Bone remodeling is balanced by the coordinated activities of osteoclastic resorption and osteoblastic formation [1]. Imbalanced bone remodeling leads to bone diseases including osteoporosis, periodontitis and rheumatoid arthritis, which are characterized by enhanced osteoclast activity. In other words, an excessive increase in osteoclast differentiation and bone resorption gives rise to various bone-resorptive diseases [2]. Osteoclasts are the cells responsible for bone resorption. These large multinucleated cells originate from the monocyte/macrophage hematopoietic lineage [3,4]. Osteoclast differentiation depends on two essential cytokines, receptor activator of nuclear factor-\textsuperscript{kB} ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) [5–7]. M-CSF is involved in the proliferation and survival of osteoclast precursors and RANKL induce osteoclast differentiation through binding to its receptor RANK and subsequent activation of nuclear factor of activated T cells (NFATc1), a master transcription factor required for osteoclast differentiation [8]. Osteoclasts are formed by the fusion of osteoclast precursor cells. Cellular fusion is an essential element in osteoclast development that results in the formation of multinucleated giant cells responsible for bone resorption activity. This process is called osteoclastogenesis. To resorb bone, osteoclasts attach to the bone surface, form a “ruffled border” and dissolve bone mineral by massive secretion of acidic elements [3].

2. The Role of Calcium (Ca\textsuperscript{2+}) Signaling in Osteoclastogenesis

Ca\textsuperscript{2+} signaling in osteoclasts is important for multiple cellular functions, including proliferation, differentiation, gene transcription and bone resorption [9]. Ca\textsuperscript{2+} is released from intracellular stores,
**3. Transient Receptor Potential (TRP) Channels in Osteoclast**

Cytosolic Ca\(^{2+}\) modulation is crucial in osteoclastogenesis. TRP channels are widely expressed in several mammalian tissues and involved in diverse physiological processes such as differentiation, proliferation, and apoptosis [27,28]. Several studies have focused on TRP channels as Ca\(^{2+}\)-influx channels in RANKL-induced osteoclastogenesis. Generally, TRP channels are non-selective cation channels and are divided into six subfamilies: canonical (TRPCs), vanilloid (TRPVs), melastatin (TRPMs), mucolipin (TRPMLs), polycystins (TRPPs), and ankyrin (TRPA) [29]. Among the TRP channels, TRPV2 [30], TRPV4 [31], and TRPV5 [32] contribute to intracellular Ca\(^{2+}\) signaling in osteoclast differentiation. TRPC1 also regulates osteoclast differentiation through SOCE [33]. This section discusses the roles of TRPC, TRPV, and TRPML channels in osteoclastogenesis.

**3.1. TRPC**

Mildly enhanced bone mass was observed in TRPC1 null mice and its effect was revealed only in mice lacking inhibitor of MyoD family isoform a (I-mfa) [33]. TRPC1 binds I-mfa [34]. Trpc1 and I-mfa functionally interact to regulate the early differentiation stage of the osteoclast through antagonistic regulation of SOCE. Although there are limited studies on TRPC, the modulation of the Ca\(^{2+}\) release-activated Ca\(^{2+}\) current (IC\(_{\text{CRAC}}\)) by TRPC1, and I-mfa is crucial for NFATc1 activation and subsequent osteoclast differentiation [33].
Figure 1. Schematic illustration of Ca\textsuperscript{2+} signaling in osteoclastogenesis. (A) RANK on the surface of osteoclast progenitor activates signaling by RANKL on the surface of osteoblasts/stromal cell to promote osteoclastogenesis. (B) Osteoclast precursor stage. In the early stages of osteoclastogenesis, RANK-bound RANKL induces activation of TRAF6 and stimulates PLC\textgamma. PLC\textgamma produces IP\textsubscript{3}, which evokes Ca\textsuperscript{2+} release from the ER. In addition, RANK-bound RANKL induces lysosomal Ca\textsuperscript{2+} release through TRPML1 and generates Ca\textsuperscript{2+} oscillation. SOCE, VGCC and TRPV2 are also involved in Ca\textsuperscript{2+} oscillation. The Ca\textsuperscript{2+} oscillations induce Ca\textsuperscript{2+}-calcineurin-NFATc1 signaling. In the late stages of osteoclastogenesis, the Ca\textsuperscript{2+} oscillation is sustained by TRPV4-mediated Ca\textsuperscript{2+} influx. In the nucleus, NFATc1 induces the expression of various osteoclast-specific genes. (C) In mature osteoclasts, TRPV4 and TRPV5 in the basolateral membrane are necessary for the regulation of osteoclastic bone resorption. TRPV5 is predominantly located on the ruffled border of resorbing osteoclasts. Abbreviations: RANKL, receptor activator of nuclear factor-\kappaB (NF-\kappaB) ligand; RANK, receptor activator of nuclear factor-\kappaB (NF-\kappaB); NFATc1, nuclear factor of activated T cells cytoplasmic 1; TRAF6, tumor necrosis factor (TNF) receptor-associated factor 6; PLC\textgamma, phospholipase C\textgamma; IP\textsubscript{3}, inositol 1,4,5-triphosphate; ER, endoplasmic reticulum; Ca\textsuperscript{2+}, calcium; [Ca\textsuperscript{2+}]\textsubscript{i}, intracellular Ca\textsuperscript{2+} concentration; SOCE, store-operated Ca\textsuperscript{2+} entry; VGCC, voltage-gated Ca\textsuperscript{2+} channel; TRPV2, transient receptor potential vanilloid 2; TRPV4, transient receptor potential vanilloid 4; TRPV5, transient receptor potential vanilloid 5; TRPML1, transient receptor potential mucolipin 1.
3.2. TRPV

TRPV family members act as sensory channels for receptor-operated Ca\(^{2+}\) influx and are critically involved in the regulating of osteoclast differentiation [20]. The TRPV family consists of six members, TRPV1–TRPV6, composed of six transmembrane domains that form a cation-permeable pore [35–37].

Among the TRPV family members, TRPV1 is a non-selective cation channel activated by various stimuli such as heat, noxious stimuli, low pH, and numerous chemicals [38]. The physiological role of TRPV1 in bone biology was addressed one decade ago. TRPV1 is expressed in osteoclasts and promotes their differentiation [39]. Human osteoclast expresses functional TRPV1, as well as the cannabinoid receptors type 1 and 2 (CB1/CB2). The involvement of both receptors is controversial. Expression levels of TRPV1 are enhanced in osteoclasts derived from osteoporotic subjects, whereas CB2 are reduced [40]. More recently, TRPV1 desensitization and/or CB2 stimulation were found beneficial for reducing osteoclast over-activity [41,42]. There are several reports showing that application of the TRPV1 agonist capsaicin suppresses LPS-induced prostaglandin E2 (PGE2) production in osteoblasts and suppressed LPS-induced osteoclast formation [39,43]. On the other hand, the TRPV1 antagonist capsazepine inhibits bone formation and bone resorption activity of osteoclasts in OVX mice [44]. [6]-Gingerol, a major constituent of ginger, augments osteoclast function via TRPV1 and induces bone loss in adult ovary-intact mice [45]. Zoledronic acid is nitrogen containing bisphosphonate that inhibit bone resorption. Effects of the Zoledronic acid were antagonized by capsazepine supporting the involvement of TRPV1 channel in osteoblastogenesis and mineralization, but this mechanism is not effective in osteoclasts lacking the TRPV1 [46]. Sirtuin 1 (SIRT1), also known as nicotinamide adenine dinucleotide (NAD\(^{+}\))-dependent lysine deacetylase, directly inhibits the osteoclast differentiation by inhibiting ROS generation and TNF-\(\alpha\)-mediated TRPV1 channel activation [47]. In addition, TRPV1, as a pain receptor, is expressed in peripheral sensory nerves [48,49]. A pathological role of TRPV1 has been revealed in both osteoporosis and osteoarthritis [41,50].

TRPV2 is closely related to TRPV1 [38,51]. TRPV2 is expressed in RANKL-treated RAW264.7 cells and TRPV2-mediated spontaneous [Ca\(^{2+}\)]\(_i\) oscillations activate NFA\(\text{Tc1}\) and promote osteoclast differentiation [30]. More recently, TRPV2 was found to regulate RANKL-dependent osteoclastic differentiation through the Ca\(^{2+}\)-calcineurin-NFATc1 signaling pathway in multiple myeloma (MM) patients [52].

TRPV4 also plays an essential role in osteoclast differentiation [31]. It is known as a mechano- and osmo-sensor [53,54], and localizes to the basolateral membranes of mature osteoclasts [31]. TRPV4-mediated Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) signaling activate NFATc1 and induce osteoclast differentiation and resorption activity [31,55]. A protein–protein interaction between TRPV4 and myosin IIa regulates Ca\(^{2+}\)/CaM signaling, which supports the migration and fusion of osteoclast precursors [55]. In addition, the TRPV4-specific antagonist, RN1734, inhibits osteoclast formation, whereas the TRPV4-specific agonist 4-\(\alpha\)-PDD enhances osteoclast formation under mild acidic conditions [56,57]. Stromal interaction molecule 1 (STIM1)-mediated SOCE is involved in fluid shear stress (FSS)-induced [Ca\(^{2+}\)]\(_i\) oscillations at the early differentiation stage of osteoclasts, whereas TRPV4 is highly associated with the Ca\(^{2+}\) response at the late stage of differentiation under FSS simulation [58]. TRPV4 knockdown significantly suppresses osteoclast differentiation and osteoporosis by inhibiting the Ca\(^{2+}\)-calcineurin-NFATc1 pathway [59].

TRPV5, a highly selective Ca\(^{2+}\) channel, is activated by low [Ca\(^{2+}\)]\(_i\) [60]. It is predominantly located on the ruffled borders of the membranes of resorbing osteoclasts [32]. TRPV5 knockout mice showed increased osteoclast numbers and reduced trabecular and cortical bone thickness [61]. In contrast, TRPV5 knockout mice had impaired osteoclastic function in vivo [32]. Although controversial, these findings suggest that TRPV5 plays an important role in osteoclastic function, again demonstrating the significance of Ca\(^{2+}\) influx in mature osteoclasts. In addition, small interfering RNA (siRNA) knockdown of TRPV5 completely inhibits RANKL-induced Ca\(^{2+}\) influx at the late differentiation stage of osteoclasts in vitro and enhances bone resorption activity in human osteoclasts [20,62]. The lack of estrogen leads to osteoporosis. Estrogen inhibits osteoclast differentiation and bone resorption activity
by increasing TRPV5 expression in postmenopausal osteoporosis [63]. Song et al. also demonstrated that estrogen increases TRPV5 expression through the interaction of the estrogen receptor α (ERα) in RAW 264.7 cells. Furthermore, NF-κB binds to the putative site on the trpv5 promoter, and TRPV5 is regulated by NF-κB [64]. Thus, TRPV5 contributes to the processes of estrogen-mediated osteoclast formation, bone resorption activity, and osteoclast apoptosis. A recent study showed that vitamin D (1,25(OH)2D3) inhibits TRPV5 expression at the early stage of osteoclastogenesis by suppressing osteoclast differentiation [65].

3.3. TRPML

The TRPML family has three members: TRPML1, TRPML2, and TRPML3. Among these, TRPML1 is a non-selective cation channel that permeates Ca2+ [66]. TRPML1 is a Ca2+-permeable channel in lysosomes and plays vital roles in lysosomal trafficking and functions [67]. Erkhembaat et al. [68] showed that deleting TRPML1 inhibits RANKL-induced [Ca2+]i oscillations, which reduces osteoclastogenesis and bone remodeling.

4. Diverse Compounds Modulating Ca2+ Signaling in Osteoclastogenesis

Osteoclasts are responsible for bone resorption and are therefore considered targets of anti-osteoporosis therapies. Novel treatment strategies aimed at preventing excessive bone resorption have been studied [69]. The study of antiresorptive agents derived from diverse compounds has become a recent topic of interest. The aim of this section is to summarize the current knowledge on diverse compounds that regulate osteoclast differentiation by modulating Ca2+ signaling. Thus, in this section, we mentioned by listing diverse compounds depending on their mode of action. Table 1 and Figure 2 summarize diverse compounds that regulate Ca2+ signaling in osteoclastogenesis.

4.1. Ca2+-Calcineurin-NFATc1 (CCN) Pathway

4.1.1. KMUP-1

KMUP-1 (7-[2-[4-(2-chlorophenyl)piperazinyl]ethyl]-1,3-dimethylxanthine), a chemical synthetic xanthine-based derivative, effectively suppresses RANKL-induced osteoclast differentiation in vitro, and also attenuated ovariectomized (OVX)-induced osteoclast differentiation and prevented bone resorption in vivo [18]. Especially KMUP-1 inhibits RANKL-induced [Ca2+]i oscillations, and subsequently, inhibits calcineurin-NFATc1 signaling [70].

4.1.2. Zinc

It has been shown that zinc, an important trace element, inhibits osteoclast differentiation by suppressing the Ca2+-calcineurin-NFATc1 signaling pathway in vitro and in vivo [19]. Specifically, zinc inhibits calcineurin activity but not expression and RANKL-induced [Ca2+]i oscillations, without decreasing PLCγ phosphorylation. In addition, it was proposed that zinc inhibits calcineurin in the early stage of osteoclast differentiation and [Ca2+]i oscillations in the middle or late stage of osteoclast differentiation [71].

4.1.3. Praeruptorin A

Praeruptorin A is isolated from the dried root of Peucedanum praeruptorum Dunn. It also has anti-osteoclastogenic activity by inhibiting [Ca2+]i oscillations without decreasing PLCγ phosphorylation [72].
Table 1. Diverse compounds that regulate Ca\(^{2+}\) signaling in osteoclastogenesis.

| Compound       | Mechanism of Inhibition of RIO \(^{(1)}\) | Species                                                                 | Administered Dose                  | Ref |
|----------------|------------------------------------------|-------------------------------------------------------------------------|-----------------------------------|-----|
| **Mode of action: Ca\(^{2+}\)-Calcineurin-NFATc1(CCN \(^{(2)}\)) signaling** |
| KMUP-1         | CCN signaling independently of PLC\(\gamma\) | RAW264.7 cell, BALB/c mice                                             | 1–10 \(\mu\)M                    | 1, 5, 10 mg/kg | [70]|
| Zinc           | CCN signaling independently of PLC\(\gamma\) | RAW264.7 cell, BMMs (C57BL/6 mice)                                      | 10–100 \(\mu\)M                  | N/A \(^{(4)}\) | [71]|
| Praeruptorin A | Inhibition of PLC\(\gamma\)-independent [Ca\(^{2+}\)], oscillations | BMMs (ICR mice)                                                         | 10 \(\mu\)M                      | N/A  | [72]|
| Cyanidin Chloride | Suppression of NF-\(\kappa\)B, ERK and CCN signaling | RAW264.7 cell, BMMs (C57BL/6 mice), C57BL/6 mice | 5–10 \(\mu\)M                  | 5 mg/kg | [73]|
| **Mode of action: PLC\(\gamma\)-Ca\(^{2+}\)-NFATc1(PCN \(^{(3)}\)) signaling** |
| Lumichrome     | Suppression of NF-\(\kappa\)B, MAPK and CCN signaling | RAW264.7 cell, BMMs (C57BL/6 mice), C57BL/6 mice | 7.5–10 \(\mu\)M                  | 7.5 mg/kg | [74]|
| Asiaticoside   | Suppression of NF-\(\kappa\)B and CCN signaling | RAW264.7 cell, BMMs (C57BL/6 mice)                                      | 2.5–20 \(\mu\)M                  | N/A  | [75]|
| **Mode of action: PLCy-Ca\(^{2+}\)-NFATc1(PCN \(^{(3)}\)) signaling** |
| OAA            | PCN signaling                              | BMMs (ICR mice), ICR mice                                              | 20 \(\mu\)M                      | 10 mg/kg | [76]|
| HAR            | Syk-Btk-PLCy- Ca\(^{2+}\) Signaling        | BMMs (ICR mice), C57BL/6 mice                                           | 25–100 \(\mu\)M                 | 10 mg/kg | [77]|
| Artesunate     | PCN signaling                              | RAW264.7 cell, BMMs (C57BL/6 mice), C57BL/6 mice                       | 3.125–12.5 \(\mu\)M             | 5, 30 mg/kg | [78]|
| MG             | Akt and Btk-PLCy- Ca\(^{2+}\) Signaling    | BMMs (ICR mice), ICR mice                                              | 1–10 \(\mu\)M                    | 10 mg/kg | [79]|
| Berberine      | * Inhibition of LPS-induced osteoclastogenesis through TRAF6 and PCN signaling | RAW264.7 cell                                                          | 5–20 \(\mu\)M                    | N/A  | [80]|
| TN             | Suppression of Btk-PLCy cascade, NF-\(\kappa\)B, MAPKs and CCN signaling | BMMs (C57BL/6 mice)                                                      | 1.25–5 \(\mu\)M                  | N/A  | [81]|
| Physalin D     | Suppression of PLCy-CaMK-CREB pathway      | BMMs (C57BL/6 mice), C57BL/6 mice                                      | 5 \(\mu\)M                      | 10, 100 mg/kg | [82]|
| Compound          | Mechanism of Inhibition of RIO (1)                                                                 | Species                  | Administered Dose | Mode of action: Negative regulation of Ca$^{2+}$ signaling | Ref |
|-------------------|-------------------------------------------------------------------------------------------------|--------------------------|-------------------|----------------------------------------------------------|-----|
| GH                | Abrogation of RANKL-induced [Ca$^{2+}$], oscillations by inactivating VGCCs independently of Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores | BMMs (C57BL/6 mice)     | 5–50 µg/mL        | N/A                                                      | [83]|
| PO                | Suppression of RANKL-induced [Ca$^{2+}$], oscillations by inhibiting Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores | murine BMMs             | 50 µg/mL          | N/A                                                      | [84]|
| MTX               | Decrease of RANKL-induced Ca$^{2+}$ influx                                                   | BMMs (C57BL/6 mice)     | 1, 5 µM           | N/A                                                      | [85]|
| XAT               | Suppression of RANKL-induced [Ca$^{2+}$], oscillations and Ca$^{2+}$-CaMKK-PyK2 signaling      | BMMs (C57BL/6 mice), C57BL/6 mice | 0.1, 1 µM, 0.5, 5 mg/kg | N/A                                                      | [86]|
| SIN               | * Inhibition of LPS-induced osteoclastogenesis by decreasing expression of NF-κB, AP-1 and Ca$^{2+}$-NFATc1 | RAW264.7 cell, C57BL/6 mice | 0.25–1 mM, 25, 50, 100 mg/kg | N/A                                                      | [87]|
| Dried plum fractions | Suppression of MAPKs and Ca$^{2+}$ signaling, resulting in inhibition of NFATc1 | RAW264.7 cell, BMMs (C57BL/6 mice) | 1, 10 µg/mL, N/A | N/A                                                      | [88]|
| KN93              | Decreasing of [Ca$^{2+}$], RAW264.7 cell                                                   | RAW264.7 cell           | 10 µM             | N/A                                                      | [89]|
| CSA               | Block of ROS activity and [Ca$^{2+}$], oscillations                                          | RAW264.7 cell, BMMs (C57BL/6 mice), C57BL/6 mice | 5–10 µM, 10 mg/kg | N/A                                                      | [90]|
| Methylglyoxal     | Suppression of [Ca$^{2+}$], mitochondrial biogenesis, mitochondrial membrane potential, and glyoxalase | RAW264.7 cell           | 10–200 µM         | N/A                                                      | [91]|
| APO               | Decreasing of [Ca$^{2+}$], BMMs (C57BL/6 mice)                                               | RAW264.7 cell           | 1 µM              | N/A                                                      | [92]|
| LrB               | Suppression of [Ca$^{2+}$], oscillations, ROS production, and NFATc1 translocation            | RAW264.7 cell, BMMs (C57BL/6 mice), C57BL/6 mice | 5–10 µM, 4 mg/kg  | N/A                                                      | [93]|
| CRT               | Suppression of RANKL-induced [Ca$^{2+}$], oscillations and expression of NFATc1 and c-Fos, independently of ionomycin-induced Ca$^{2+}$ influx | RAW264.7 cell, BMMs (C57BL/6 mice), C57BL/6, NOD mice | 0.5–500 ng/ml, 0.2 mg/kg | N/A                                                      | [94]|
| 6-Shogaol         | Suppression of [Ca$^{2+}$], oscillations, ROS production, and NFATc1 activity                 | BMMs (C57BL/6 mice), C57BL/6 mice | 2.5–10 µM, 10 mg/kg | N/A                                                      | [95]|
|                  | * Enhancement of osteoclast activation by activating NF-κB, ERK and increasing [Ca$^{2+}$], oscillations, resulting in upregulation of NFATc1 | BMMs (C57BL/6 mice)     | 1–10 µM           | N/A                                                      | [96]|

* Another mechanism besides RIO, Abbreviations: (1) RIO, RANKL-induced osteoclastogenesis; (2) CCN, Ca$^{2+}$-Calcineurin-NFATc1; (3) PCN, PLCγ- Ca$^{2+}$-NFATc1; (4) N/A, not applicable; The other abbreviations are listed in the last paragraph.
Osteoclastogenesis

Figure 2. The schematic illustration summarized diverse compounds that regulate Ca\(^{2+}\) signaling in osteoclastogenesis. KMUP-1 (7-[2-[4-(2-chlorophenyl)piperazinyl]ethyl]-1,3-dimethylxanthine), Zinc, Praeruptorin A, Cyanidin Chloride, Lumichrome and Asiaticoside inhibit osteoclastogenesis via inhibiting Ca\(^{2+}\)-Calcineurin-NFATc1 signaling independent of PLC\(\gamma\). Methotrexate (MTX), Xanthotoxin (XAT), Sinomenine (SIN), Dried plum fractions, KN93, Cajaninstilbene acid (CSA), Methylglyoxal, Apocynin (APO), Loureirin B (LrB), Calreticulin (CRT) and 6-Shogaol inhibit osteoclastogenesis via decreasing [Ca\(^{2+}\)]. On the contrary, Amyloid beta peptide (A\(\beta\)) enhances osteoclastic bone resorption by increasing [Ca\(^{2+}\)], oscillations, resulting in upregulation of NFATc1. Portalaca oleracea (PO) inhibits osteoclastogenesis by inhibiting Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores. Oleanolic acid acetate (OAA), Artesunate, Berberine and Physalin D inhibit osteoclastogenesis via inhibiting PLC\(\gamma\)-Ca\(^{2+}\)-NFATc1 signaling. Harpagoside (HAR) inhibits osteoclastogenesis via inhibiting Syk-Btk-PLC\(\gamma\)-Ca\(^{2+}\) Signaling. Methyl gallate (MG) and Tatarinan N (TN) inhibit osteoclastogenesis by suppression of Btk-PLC\(\gamma\) cascade. Glechoma hederacea (GH) inhibits osteoclastogenesis by inactivating VGCCs independent of Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores. Abbreviations: RANKL, receptor activator of nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) ligand; RANK, receptor activator of nuclear factor-\(\kappa\)B (NF-\(\kappa\)B); NFATc1, nuclear factor of activated T cells cytoplasmic 1; TRAF6, tumor necrosis factor (TNF) receptor-associated factor 6; MAPK, mitogen-activated protein kinases; AP-1, activator protein-1; Btk, Bruton’s tyrosine kinase; Syk, spleen tyrosine kinase; PLC\(\gamma\), phospholipase C\(\gamma\); IP3R, inositol 1,4,5-triphosphate receptor; ER, endoplasmic reticulum; Ca\(^{2+}\), calcium; [Ca\(^{2+}\)], intracellular Ca\(^{2+}\) concentration; SOCE, store-operated Ca\(^{2+}\) entry; VGCC, voltage-gated Ca\(^{2+}\) channel; TRP channels, transient receptor potential cation channels; CaMKs, Ca\(^{2+}\)/calmodulin dependent protein kinases; CREB, cAMP-responsive element-binding protein; CaM, calmodulin.
4.1.4. Cyanidin

Cyanidin, a particular type of anthocyanidins, is the sugar-free counterpart of anthocyanins. Anthocyanins are reddish pigments widely spread in colored fruits and vegetables [97,98]. Cyanidin chloride inhibits RANKL-induced osteoclast formation and osteoclast resorptive activity in vitro and protects against OVX-induced bone loss in vivo. Furthermore, cyanidin chloride impairs RANKL-induced \([Ca^{2+}]\) oscillations, which leads to the suppression of the activation of NFATc1 in cultured primary bone marrow-derived macrophages (BMMs) [73]

4.1.5. Lumichrome

Lumichrome is a natural metabolite of riboflavin, a member of the B family of vitamins, and has been shown to have a beneficial effect on bone formation [99,100]. Chuan et al. [74] found that lumichrome inhibits RANKL-induced \([Ca^{2+}]\) oscillations in BMMs. Furthermore, lumichrome suppresses NFATc1, NF-\(\kappa\)B, and MAPK signaling activation and decreases bone loss in OVX-mice by inhibiting osteoclastogenesis.

4.1.6. Asiaticoside

Asiaticoside, a natural compound, is extracted from Centella asiatica and is a member of the triterpenoid family [101]. It significantly inhibits RANKL-induced \([Ca^{2+}]\) oscillations in BMMs. Therefore, Asiaticoside suppresses the differentiation and function of the osteoclast via inhibiting the NF-\(\kappa\)B and NFATc1 pathways [75].

4.2. PLC\(\gamma\)-Ca\(^{2+}\)-NFATc1 (PCN) Pathway

4.2.1. Oleanolic Acid Acetate

Oleanolic acid acetate (OAA) is a compound isolated from Vigna angularis (azuki bean). Kim et al. [76] have reported that OAA negatively regulates osteoclast differentiation by RANKL-induced PLC\(\gamma\)2 and \([Ca^{2+}]\) oscillations, which leads to NFATc1 activation. In vitro, OAA inhibits RANKL-induced osteoclast differentiation through PLC\(\gamma\)2-Ca\(^{2+}\)-NFATc1 signaling. OAA administration also suppresses lipopolysaccharide (LPS)-induced bone loss in vivo.

4.2.2. Harpagoside

Harpagoside (HAR), an iridoid glycoside isolated from Harpagophytum procumbens (devil’s claw), inhibits \([Ca^{2+}]\) oscillations via inactivation of several kinases such as Bruton’s tyrosine kinase (Btk), spleen tyrosine kinase (Syk), and PLC\(\gamma\)2, which leads to the suppression of RANKL-induced osteoclast differentiation [77]. HAR also restored bone density in an LPS-induced, but not in an OVX-induced bone loss mouse model in vivo [77].

4.2.3. Artesunate

Artesunate is one of the effective clinical treatments for falciparum malaria [102]. It suppresses RANKL-induced Ca\(^{2+}\) influx and calcineurin expression. Furthermore, phosphorylation of PLC\(\gamma\)1 is decreased by artesunate treatment in RANKL-stimulated RAW264.7 cells. Therefore, artesunate suppresses RANKL-induced osteoclast differentiation and function by inhibiting the PLC\(\gamma\)1-Ca\(^{2+}\)-calcineurin-NFATc1 pathway [78].

4.2.4. Methyl Gallate

Methyl gallate (MG) is a polyphenolic compound that is known to have antioxidant [103], antitumor [104], anti-inflammatory [105], and antimicrobial activities [106]. MG is a dominant inhibitor of sodium and potassium ion channels in skeletal muscle cells [107]. Baek et al. [79] showed that
MG attenuates RANKL-induced osteoclast differentiation by inhibiting both Akt (Protein kinase B) phosphorylation and intracellular Ca\(^{2+}\) influx mediated by Btk and PLC\(\gamma\)2.

### 4.2.5. Berberine Hydrochloride

Berberine hydrochloride, an isoquinoline alkaloid, is found in many plants of the Berberidaceae families [108]. It inhibits the activation of PLC\(\gamma\)1, and thereby, inhibits Ca\(^{2+}\) influx, which reduces intracellular Ca\(^{2+}\) concentration, and subsequently, inhibits osteoclast differentiation and bone destruction through suppression of the TRAF6-Ca\(^{2+}\)-calcineurin-NFATc1 signaling pathway in LPS-stimulated RAW264.7 cells [80].

### 4.2.6. Tatarinan N

Tatarinan N (TN), a lignin-like component, is extracted from *Acorus tatarinowii Schott* [109]. It attenuates RANKL-induced osteoclast differentiation via reducing NFATc1 and c-Fos expression as well as inhibiting the ERK1/2 or p38 signaling pathway. Besides, TN significantly reduces the elevation of intracellular Ca\(^{2+}\) concentration induced by RANKL and attenuates RANKL-induced phosphorylation of Btk and PLC\(\gamma\)2 in a dose-dependent manner in BMMs [81].

### 4.2.7. Physalin D

Physalin D is isolated from *Physalis alkekengi* L., known as “winter cherry”, and grows in western Asia and Europe [110]. Physalin D has been shown to have anti-inflammatory, antimalarial, and antinociceptive effects [110–112]. Physalin D attenuates RANKL-induced [Ca\(^{2+}\)]\(_i\) oscillations by inhibiting phosphorylation of PLC\(\gamma\)2 and blocks the downstream activation of Ca\(^{2+}\)/calmodulin-dependent protein kinase (CaMK) type IV and cAMP-responsive element-binding protein (CREB) in BMMs. Moreover, physalin D protects RANKL-induced bone loss in vivo [82].

### 4.3. Negative Regulation on Ca\(^{2+}\) Signaling

#### 4.3.1. Glechoma Hederacea

*Glechoma hederacea* (GH), known as ‘ground ivy’ or ‘creeping Charlie’, is a perennial hairy herb of the mint family Lamiaceae. Hwang et al. [83] have shown that GH induces a transient and large increase in [Ca\(^{2+}\)]\(_i\), through the involvement of Ca\(^{2+}\) influx via voltage-gated Ca\(^{2+}\) channels (VGCCs), resulting in the abrogation of RANKL-induced [Ca\(^{2+}\)]\(_i\) oscillations and the inhibition of NFATc1 expression in BMMs. However, GH-induced intracellular [Ca\(^{2+}\)]\(_i\) elevation was independent of Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores in BMMs. Taken together, these findings suggest that GH abrogates RANKL-induced [Ca\(^{2+}\)]\(_i\) oscillations, inhibits NFATc1 expression, and reduces osteoclast differentiation by inactivating VGCCs.

#### 4.3.2. Portulaca Oleracea

*Portulaca oleracea* (PO), also known as verdolaga, red root, or pursley, has been widely used as traditional medicine. PO ethanol extract (POEE) has dual and contrary effects on RANKL-induced osteoclast differentiation. The POEE inhibits RANKL-induced [Ca\(^{2+}\)]\(_i\) oscillations and NFATc1 activation, while it enhances RANKL-induced osteoclast differentiation by reducing RANKL-mediated cytotoxicity. Erkhembaatar et al. [84] proposed that RANKL-mediated cytotoxicity due to Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores is attenuated by POEE, which leads to enhanced RANKL-induced osteoclast differentiation.
4.3.3. Methotrexate

Methotrexate (MTX) is used to treat sarcoma, leukemia, and auto-inflammatory diseases such as rheumatoid arthritis [113,114]. MTX inhibits osteoclast differentiation by inhibiting RANKL-induced Ca^{2+} influx in osteoclast progenitor cells [85].

4.3.4. Xanthotoxin

Xanthotoxin (XAT) is isolated from the seeds of a plant of the carrot family Ammi majus [115]. XAT has been shown to have antitumor activity and antioxidant activity [116,117]. Interestingly, XAT affects the intracellular Ca^{2+} levels in melanocytes, resulting in reorganization of actin stress fiber cytoskeleton [118]. Dou et al. [86] showed that XAT suppresses RANKL-induced [Ca^{2+}]_i oscillations and the activation of downstream targets of Ca^{2+}-CaMKK (Calmodulin-dependent protein kinase kinase)/Pyk2 (Proline-rich tyrosine kinase 2) signaling during osteoclast differentiation, resulting in the inhibition of NFATc1 and c-FOS in BMMs. In addition, an in vivo study showed that XAT treatment prevents bone loss and increases new bone formation in OVX-mice.

4.3.5. Sinomenine

Sinomenine (SIN) is an alkaloid found in the roots and stems of Sinomenium acutum. SIN has been used for the treatment of rheumatoid arthritis (RA) in China [119]. SIN dramatically reduces LPS-induced upregulation of intracellular Ca^{2+} in matured RAW264.7 cells. In addition, SIN decreases expression of osteoclast-specific genes and tumor necrosis factor-α (TNF-α) production, and inhibits LPS-induced osteolysis and osteoclast differentiation in vitro and in vivo [87].

4.3.6. Dried Plum Fractions

In preclinical trials, bone resorption is decreased by dietary supplementation with dried plum in ovariectomized rat and mouse models [120,121]. Graef et al. [88] showed that polyphenolic compounds in dried plums suppress intracellular Ca^{2+} signaling and MAPK signaling, resulting in the inhibition of NFATc1 expression, which reduces osteoclast differentiation in BMMs.

4.3.7. KN93

KN93 is an inhibitor of multifunctional Ca^{2+}/CaMKs [122]. It inhibits the formation and activation of the osteoclast. KN93 also downregulates the expression of NFATc1 and AP-1 protein family members in RANKL-stimulated RAW 264.7 cells. Furthermore, KN93 significantly decreases intracellular Ca^{2+} concentration in differentiated osteoclasts [89].

4.3.8. Cajaninstilbene Acid

Cajaninstilbene acid (CSA) is a bioactive compound derived from pigeon pea leaves [123]. It suppresses osteoclast differentiation and bone resorption via inhibiting RANKL-induced ROS activity and [Ca^{2+}]_i oscillations in RAW264.7 cells and BMMs. CSA also protects the bone loss of OVX-induced C57BL/6 mice [90].

4.3.9. Methylglyoxal

Methylglyoxal is derived from organic compounds and is a precursor of advanced glycation end products. Its formation involves several metabolic pathways [124]. The formation of Methylglyoxal is increased in diabetic patients [125]. Diabetes can give rise to a state of low bone turnover osteoporosis [126]. The Methylglyoxal decreases [Ca^{2+}]_i, mitochondrial biogenesis, mitochondrial membrane potential, and glyoxalase I, resulting in the inhibition of RANKL-induced osteoclast differentiation and bone resorbing activity in RAW264.7 cells [91].
4.3.10. Apocynin

The catechol apocynin (APO) is used as a NADPH oxidase (NOX) inhibitor [127]. Soares et al. [92] evaluated the effects of APO on osteoclast differentiation. APO reduces $[Ca^{2+}]_i$ by blocking $Ca^{2+}$ channels except two pore segment channel 2 (TPC2) and inositol 1,4,5-triphosphate receptor type 1 (IP$_3$R1). TPC2 is a $Ca^{2+}$-permeable channel expressed in lysosomes, and IP$_3$R1 is a $Ca^{2+}$ channel that mediates $Ca^{2+}$ release from the ER, following IP$_3$ stimulation. APO inhibits osteoclast differentiation by decreasing $[Ca^{2+}]_i$.

4.3.11. Loureirin B

Loureirin B (LrB) is an active component isolated from Sanguis draxonis, which is a Chinese traditional herb also known as Dragon’s Blood [128]. Yuhao et al. [93] investigated the effects of LrB on RANKL-induced osteoclast activity in vitro and in an OVX-induced osteoporosis mouse model in vivo. LrB attenuates RANKL-induced $[Ca^{2+}]_i$, oscillations, ROS production, and NFATc1 translocation into the nucleus in BMMs. Therefore, LrB can inhibit osteoclast differentiation and function by suppressing $[Ca^{2+}]_i$, oscillations, ROS, and NFATc1 activities. LrB also exerts a protective effect on OVX-induced osteoporosis in a mouse model [93].

4.3.12. Calreticulin

Calreticulin (CRT) is a $Ca^{2+}$-binding protein that regulates intracellular $Ca^{2+}$ homeostasis by modulating cytoplasmic and ER $Ca^{2+}$ levels [129–131]. Fischer et al. [94] found that exogenous CRT has an anti-osteoclastogenic effect in vitro and in vivo. Recombinant CRT Inhibits RANKL-induced $[Ca^{2+}]_i$ oscillations, but not ionomycin-induced $Ca^{2+}$ influx in BMMs. Recombinant CRT also blocks expression of NFATc1 and c-Fos, but not CREB and NF-$\kappa$B in RAW264.7 cells.

4.3.13. 6-Shogaol

Shogaols are significant biomarkers used for the quality control of ginger-containing products and responsible for the pungent flavor in dried ginger. Among them, 6-shogaol is the most common type [132]. The 6-shogaol inhibits RANKL-induced $[Ca^{2+}]_i$ oscillations, ROS production, and NFATc1 activities in BMMs. Furthermore, 6-shogaol attenuates osteoclastogenesis and alveolar bone resorption in a ligature-induced periodontitis model in vivo [95].

4.4. Increasing $[Ca^{2+}]_i$ Oscillations

Amyloid Beta Peptide

Amyloid beta peptide (A$\beta$) is the principal component of the accumulations of $\beta$-amyloid found in the brains of Alzheimer’s patients [133]. Various studies have addressed the role of A$\beta$ in osteoclasts [134–136]. Specifically, a recent study showed that A$\beta$ enhances RANKL-induced osteoclast activation and functions through nuclear factor-$\kappa$B inhibitor $\alpha$ (I$\kappa$B-$\alpha$) degradation, extracellular-signal-regulated kinase (ERK) phosphorylation, and increased $[Ca^{2+}]_i$ oscillations in BMMs [96].

5. Closing Remarks and Perspectives

The crucial studies on $Ca^{2+}$ signaling in osteoclastogenesis have highlighted its role in bone biology. Considering the involvement of $Ca^{2+}$ signaling in bone biology, the relatively few studies available to date suggest the importance of TRP channels for modulating osteoclastogenesis and bone loss. Therefore, most of the therapeutic potentials remain open. We estimate that pharmacological targeting of this membrane channels may result in the development of therapeutics that facilitate or inhibit $Ca^{2+}$ influx.
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Abbreviations

RANKL receptor activator of nuclear factor-κB (NF-κB) ligand
M-CSF macrophage colony-stimulating factor
NFATc1 nuclear factor of activated T cells
Ca\(^{2+}\) calcium
TRAF6 tumor necrosis factor (TNF) receptor-associated factor 6
MAPKs mitogen-activated protein kinases
NF-κB nuclear factor-κB
AP-1 activator protein-1
PLC\(\gamma\) phospholipase \(\gamma\)
IP\(_3\) inositol 1, 4, 5-triphosphate
\([\text{Ca}\(^{2+}\)]\(_i\)\) intracellular \(\text{Ca}^{2+}\) concentration
ER endoplasmic reticulum
SOCE store-operated \(\text{Ca}^{2+}\) entry
TRP transient receptor potential
SERCA Sarco/endothelial \(\text{Ca}^{2+}\)-ATPase
ROS reactive oxygen species
CaM calmodulin
Homer2/3 Homer2 and Homer3
DKO double-knockout
TRPCs Transient receptor potential canonical channel
TRPVs Transient receptor potential vanilloid channel
TRPMs Transient receptor potential melastatin channel
TRPMLs Transient receptor potential mucolipin channel
TRPPs Transient receptor potential polycystin channel
TRPAs Transient receptor potential ankyrin channel
I-mfa inhibitor of MyoD family isoform \(a\)
I\(_{\text{CRAC}}\) \(\text{Ca}^{2+}\) release-activated \(\text{Ca}^{2+}\) current
CB1/CB2 cannabinoid receptors type 1 and 2
PGE2 prostaglandin \(E2\)
SIRT1 Sir2 protein 1
MM multiple myeloma
STIM1 Stromal interaction molecule 1
FSS fluid shear stress
siRNA small interfering RNA
ER\(\alpha\) estrogen receptor \(\alpha\)
CCN \(\text{Ca}^{2+}\)-Calcineurin-NFATc1
OVX ovariectomized
BMMs bone marrow–derived macrophages
OAA Oleanolic acid acetate
LPS lipopolysaccharide
HAR Harpagoside
Btk Bruton’s tyrosine kinase
Syk spleen tyrosine kinase
MG Methyl gallate
TN Tatarinan N
CaMK  CaMKII, calmodulin-dependent protein kinase
CREB  cAMP-responsive element-binding protein
GH  Glechoma hederacea
VGCCs  voltage-gated Ca\(^{2+}\) channels
PO  Portulaca oleracea
POEE  Portulaca oleracea ethanol extract
MTX  Methotrexate
XAT  Xanthotoxin
CaMKK  Calmodulin-dependent protein kinase kinase
Pyk2  Proline-rich tyrosine kinase 2
SIN  Sinomenine
RA  rheumatoid arthritis
TNF-\(\alpha\)  tumor necrosis factor-\(\alpha\)
CSA  Cajaninstilbene acid
APO  Apocynin
NOX  NADPH oxidase
TPC2  two pore segment channel 2
IP\(_3\)R1  inositol 1,4,5-triphosphate receptor type 1
LrB  Loureirin B
CRT  Calreticulin
A\(\beta\)  Amyloid beta peptide ()
I\(k\)B-\(\alpha\)  nuclear factor-\(k\)B inhibitor \(\alpha\)
ERK  extracellular-signal-regulated kinase

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