REVIEW ARTICLE

Regulation of Bone Resorption by Osteoclasts- an Overview

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ABSTRACT

Osteoporosis is related to estrogen deficiency and aging. Bone loss can also occur as a result of inflammation-associated diseases such as rheumatoid arthritis and periodontitis, which share several pathologic features with osteoporosis. Estrogen deficiency is associated with increased osteoclast activation, decreased osteoblast function, and increased inflammatory bone-resorbing cytokines (e.g., interleukin-1, -6, and tumor necrosis factor –α). The differentiation of osteoclasts is regulated by the cytokines macrophage colony-stimulating factor, RANK ligand, and osteoprotegerin secreted by osteoblasts. Bone resorption is the unique function of osteoclasts. Podosomes are essential features of osteoclast migration. Podosomes are F-actin rich structures joined radially by actin fibers called F-actin cloud. Upon attachment to the bone surface, osteoclasts reorganize their cytoskeleton to form sealing zones. Sealing zone formation is required for efficient bone resorption to occur by osteoclasts. Integrin αvβ3 and TNF-alpha mediated signaling mechanisms regulate the assembly/disassembly of podosomes during migration and the organization of sealing zones during bone resorption. A brief description is provided on these aspects in this review.

1.Introduction

Osteoporosis is a disease caused by the imbalanced activities of bone cells such as osteoclasts and osteoblasts. Osteoclasts mediate bone resorption or erosion, and osteoblasts facilitate the bone formation. Osteoclasts in excessive numbers or increased activity cause a faster rate of loss of bone than the action of osteoblasts to rebuild bone, which puts individuals at risk for fracture. Excess bone loss leads to pathological postmenopausal osteoporosis or bone-metastasis associated bone loss in cancer patients. Anti-osteoporosis therapy up to now has targeted mostly osteoclasts. Low bone mass is caused by an increase in the recruitment and activity of osteoclasts. The precise cause of osteoporosis is not entirely known. However, there is a relationship between the lack of estrogen due to ovarian failure and the onset of loss of bone mass. Furthermore, estrogen deficiency in women also increases the production of inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-α) from T-cells to a level sufficient to modify the formation and function of bone-resorbing osteoclasts. Therefore, the neutralization of these cytokines could protect the bone loss caused by estrogen deprivation [1].

2. Bone cells and osteoclastogenesis

Osteoclasts are multinucleated giant cells that are responsible for bone resorption. These cells are derived from hematopoietic precursors or stem cells in the monocyte/macrophage lineage. The differentiation process is known as 'osteoclastogenesis' is mediated by two key cytokines, such as Macrophage Colony-Stimulating Factor-1 (M-CSF) and the Receptor Activator of Nuclear factor-kB Ligand (RANKL). Osteoblasts are derived from mesenchymal stem cells from the bone marrow and periosteum of the bone. These cells are involved in the formation and mineralization of bones. Osteoblasts then mature into osteocytes, which do not form bone matrix. Osteoblasts secrete cytokines such as M-CSF1 and RANKL besides osteoprotegerin (OPG), which are involved in osteoclastogenesis. RANKL belongs to the tumor necrosis factor family, and the Receptor Activator of Nuclear factor-kB (RANK) is the receptor for RANKL. OPG is a soluble decoy receptor for RANKL, and it is also considered a critical regulator of osteoclastogenesis. OPG functions to reduce osteoclastogenesis and osteolysis by competitively binding to RANKL and blocks the interaction of RANKL with its receptor RANK. The process of osteogenesis ensues with the balancing between the activities of osteoblasts (bone formation) and osteoclasts (bone resorption).

3. Podosomes and sealing zones in osteoclasts

The bone-resorbing function of osteoclasts is dependent on actin cytoskeletal remodeling. Osteoclasts are uniquely and profoundly migratory. They depend on rapid changes in their actin cytoskeleton to undertake the controlled cycles of migration and adhesion on the bone surface during bone resorption. Osteoclasts depend on podosomes for attachment and movement on the bone surface. Podosomes are found in highly motile cells, and these are comparable to focal adhesion structures. Although podosomes and focal adhesions are different morphologically and functionally, some functional differences exist. Podosomes are highly dynamic structures with a life-span of 2-12 min compared with 30min for the focal adhesions [2]. Podosomes are dot-like aggregates with packed filamentous actin (F-actin) surrounded by a globular monomeric form of actin (G-actin) [3]. These dot-

like podosomes cluster in a ring around the cell periphery [4].

On the bone surface, osteoclasts exhibit a continuous band of F-actin rich ring-like structures called sealing zones, which confine the area of bone resorption. Loss of peripheral podosome structures occurs in resorbing osteoclasts. Under these conditions sealing zones provide a tight attachment. Researchers denoted these structures as sealing zones, actin rings or sealing rings [13-20]. Sealing zone formation has been considered a marker of osteoclast activation for bone resorption.

One crucial feature of podosomes is their dynamic nature, and they are rapidly constructed and removed. Sealing zones have very stable adhesion to ECM on the bone surface. It is not completely known whether sealing zones are derived from podosomes. It is mostly presumed that towards the resorption phase, the sealing zones are derived from the fusion of podosomes [21-23]. However, various findings suggest that the sealing zones have a unique three-dimensional organization on the bone. Podosomes do not fuse to form an actin ring onto the dentine slice or mineralized matrix [24, 25]. Osteoclasts are multinucleated giant cells. An osteoclast can do resorption of the bone underneath one area of the cell, and at the same time, another area of the cell can organize podosomes [5].

Gelsolin is an actin capping protein, and it is present in the podosomes of osteoclasts. It has a unique role in regulating the assembly and disassembly of the actin filaments present in the podosomes of osteoclasts through its binding to polyphosphoinositides. Osteoclasts derived from gelsolin knockout mice failed to exhibit podosomes. However, these osteoclasts still revealed sealing zones and matrix resorption in vitro; however, the resorbed areas are small as a result of the absence of podosomes and the hypomotile nature of osteoclasts [11]. Studies in gelsolin knockout mice indicate that podosomes do not fuse to form the sealing zones. Several questions about the mechanisms involved in sealing zone formation remain unanswered. Does sealing zone formation require a progression from podosomes? What is the mechanism of initiation of the organization of sealing zone formation?

4. Role of integrin αvβ3 signaling in osteoclast function

Integrin alpha v beta 3 (αvβ3) is the principal osteoclast integrin. It recognizes the Arg-Gly-Asp (RGD) motif of the extracellular matrix proteins located on the bone surface. Osteoclasts from β3 knockout (β3-/-) mice failed to form sealing zones on the bone matrix during resorption in vitro. These mice demonstrated an age-dependent increase in bone mass, consistent with the dysfunction of osteoclasts [27, 28]. Integrin signaling, which involves different pathways and molecules (e.g., Src, PYK2, c-Cbl, p130Cas, PTP–PEST, Vav, PI3-kinase, and WASP) have been shown to participate in the formation of the sealing zones during bone resorption [12, 19, 29-38]. Src has been implicated as a crucial downstream target of integrin signaling in osteoclast function [19, 39-42]. Osteoclasts from c-Src kinase knockout (Src-/-) mice shared several features with β3-/-osteoclasts [43]. Although Src-/- mice exhibited an increase in osteoclast number, these mice develop severe osteopetrosis due to dysfunctional bone resorption. Osteoclasts from Src-/- mice do not form sealing zones. Similar cytoskeletal arrangements in osteoclasts isolated from c-Src-/- and β3-/- mice suggest a commonality of intracellular signaling pathways. PYK2 is a calcium-sensitive protein tyrosine kinase, and it belongs to a member of the focal adhesion kinase (FAK) family of protein

kinases. C-src kinase activity and targeting of C-Src kinase by PYK2 are essential for the cytoskeletal reorganization involved in osteoclastic bone resorption in response to αvβ3 signaling [6, 44].

Many actin-binding proteins (ABPs) assist in stabilizing and rearranging the organization of the actin cytoskeleton during cell migration and adhesion. A few ABPs appear to have a role in osteoclast function. Spatial configurations of actin filaments by actin-binding and actin regulatory proteins account for the highly specific changes in cell shape during migration and bone resorption [18, 19, 33, 35, 45-52]. Rho family GTPases have been shown to be essential in the formation and organization of podosomes as well as for polarization of osteoclasts on the bone surface during bone resorption [30, 35, 47, 53-56]. Rac1 and Rac2 are involved in the formation of the podosome belt and bone resorption besides Rho A [57-59]. Rac1- and Rac2-deficient osteoclasts exhibited reduced resorption activity[59]. Overall studies by these groups imply the essential role of RhoGTPase modulators on the function of osteoclasts.

5. Role of TNF-alpha signaling in osteoclast function

RANKL is an essential and sufficient cytokine for osteoclast differentiation in the presence of macrophage colony-stimulating factor (M-CSF). However, proinflammatory factors such as TNF-α and interleukin-1 (IL-1) can also promote osteoclast differentiation and bone loss in postmenopausal osteoporosis and pathological conditions due to inflammation (e.g., rheumatoid arthritis and periodontitis) [1, 60-67]. TNF-α stimulates osteoclast differentiation and resorption activity independent of the RANK- osteoclast differentiation factor (ODF) interaction in chronic inflammatory diseases [68-70]. TNF-α directly activates the formation of sealing zones in osteoclasts formed *in vivo* or *in vitro* [70]. The process of sealing zone formation requires a significant reorganization of actin filaments. Actin filaments generate tight sealing zones on the bone surface for efficient bone resorption processes to occur. The area encompassed by actin filaments in the sealing zone area ranges from 1-10μm. A dramatic increase in the local levels of F-actin and actin-binding proteins were found during the transformation of clustered podosomes into ring-like sealing zone structures [71]. Even though the formation of these actin filament-associated sealing zone is indispensable for osteoclast bone resorption, there is a shortage of information on the cell and molecular biology of the formation of sealing zones.

6. Role of L-plastin in osteoclast bone resorption

L-plastin (LPL) is an actin-bundling protein that cross-links actin filaments into tight bundles, [72-74]. LPL is also known as plastin-2, fimbrin, or cytoskeletal associated protein [75]. It was shown to present in the podosomes of osteoclasts [7]. However, its role in osteoclasts was largely unknown. LPL consists of two tandem repeats of actin-binding domains (ABDs), which mediate the bundling of the actin filament. These ABDs assist in binding two actin filaments into parallel arrays for bundling assembly [77-79]. Phosphorylation of LPL on Serine 5 and Serine 7 amino acid (aa) residues regulate the actin-bundling activity of LPL [8]. Although TNF-α was shown to stimulate the resorptive activity of osteoclasts [68, 81], the actual target molecule(s) involved in the organization of sealing zones remains unknown.

In our recent studies, we have shown the role of LPL in the formation of the precursor sealing zones in osteoclasts cultured on dentine slices in the presence of TNF-α. These precursor zones were denoted as 'nascent sealing zones' (NSZs). We demonstrated previously that sealing zone formation occurs in two steps: the first step is the formation of an NSZ, which is regulated by the phosphorylation of LPL in a TNF-α–dependent way. The second step is the transition from nascent sealing zones to fully functional sealing rings, which is controlled by integrin αvβ3 signaling. Several studies demonstrated the role of integrin αvβ3 in the formation of sealing zones. However, we are the first one to elucidate the role of LPL in NSZ formation in response to TNF-α signaling independently of integrin signaling in osteoclasts [82].

Serine phosphorylation of LPL in response to TNF-α treatment is a necessary process in the actin-bundling process involved in the organization of NSZs. We suggest this because the transduction of a small molecular peptide of LPL containing Ser-5 and Ser-7 amino acid residues [*MARGSVSDEE; 10aa] into osteoclasts suppressed the phosphorylation of endogenous LPL protein competitively. Therefore, the formation of NSZs and hence the formation of mature sealing zones were attenuated, which resulted in reduced bone resorption in vitro [83-85]. [Note: *patent pending for the small molecular peptide of LPL].

Furthermore, analyses in osteoclasts from L-plastin knockout (LPL -/-) mice have shown a defect in the formation of the sealing zones, which is associated with an increase in trabecular bone volume and a decrease in eroded perimeters, indicating a mild osteopetrosis phenotype [20]. Consistent with our observations, others have also shown an increase in trabecular bone volume in LPL-/- mice [86]. Studies with small molecular weight LPL peptide and LPL-/- mice suggest that LPL phosphorylation is a potential mechanism in the formation of NSZs by LPL. The Abrogation of LPL phosphorylation and the formation of NSZs is a unique approach to block osteoclast-mediated bone resorption.

7. Conclusions

The regulation of the assembly of NSZs by TNF-α signaling is a prerequisite for the formation of sealing zones, which are required for bone resorption by osteoclasts. Excess osteoclast activity puts individuals at risk for fracture in bone loss-associated diseases, including osteoporosis, rheumatoid arthritis, and periodontitis. Although targeted therapies are currently available to treat and prevent osteoporosis by blocking osteoclast activity, evidence shows that long-term treatments cause a reduction in osteoblast-mediated bone formation, resulting in atypical skeletal fractures. The distinctiveness of the LPL peptide is its targeted effect on osteoclasts without interfering with the function of osteoblasts. We suggest that the small molecular weight LPL peptide has the potential for high translational control in the progression of physiological and pathological bone loss. The role of LPL in osteoclast sealing ring formation is unique because LPL-/- mice failed to form these structures. Other plastin proteins or actin-binding proteins cannot substitute LPL's functions of actin-bundling and NSZ formation in LPL-/- mice. Moreover, peptides of LPL could be used in protein therapy for modulation of osteoclast bone resorption. LPL based signaling complex is an attractive target for pharmacological regulation of bone resorption.

9. References

1) Cenci S, Weitzmann MN, Roggia C et al. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. *J Clin Invest* 2000;106:1229-1237.
2) Boyce BF, Li P, Yao Z et al. TNF-alpha and pathologic bone resorption. *Keio J Med* 2005;54:127-131.
3) Roggia C, Tamone C, Cenci S, Pacifici R, Isaia GC. Role of TNF-alpha producing T-cells in bone loss induced by estrogen deficiency. *Minerva Med* 2004;95:125-132.
4) Charatcharoenwitthaya N, Khosla S, Atkinson EJ, McCready LK, Riggs BL. Effect of blockade of TNF-alpha and interleukin-1 action on bone resorption in early postmenopausal women. *J Bone Miner Res* 2007;22:724-729.
5) Kanehisa J, Yamanaka T, Doi S et al. A band of F-actin containing podosomes is involved in bone resorption by osteoclasts. *Bone* 1990;11:287-293.
6) Akisaka T, Yoshida H, Inoue S, Shimizu K. Organization of cytoskeletal F-actin, G-actin, and gelsolin in the adhesion structures in cultured osteoclast. *J Bone Miner Res* 2001;16:1248-1255.
7) Pfaff M, Jurdic P. Podosomes in osteoclast-like cells: structural analysis and cooperative roles of paxillin, proline-rich tyrosine kinase 2 (Pyk2) and integrin alphaVbeta3. *J Cell Sci* 2001;114:2775-2786.
8) Marchisio PC, Cirillo D, Naldini L, Primavera MV, Teti A, Zambonin-Zallone A. Cell-substratum interaction of cultured avian osteoclasts is mediated by specific adhesion structures. *J Cell Biol* 1984;99:1696-1705.
9) Zhang D, Murakami H, Udagawa N et al. The small GTP-binding protein *Rho* is involved in osteoclastic bone resorption by regulating podosome formation. *J Bone Miner Res* 1994;9:S131.
10) Chellaiah M, Fitzgerald C, Alvarez U, Hruska K. C-*src* is required for stimulation of gelsolin associated PI3-K. *J Biol Chem* 1998;273:11908-11916.
11) Chellaiah M, Kizer N, Silva M, Alvarez U, Kwiatkowski D, Hruska KA. Gelsolin deficiency blocks podosome assembly and produces increased bone mass and strength. *J Cell Biol* 2000;148:665-678.
12) Chellaiah M, Soga N, Swanson S et al. Rho-A is critical for osteoclast podosome organization, motility, and bone resorption. *J Biol Chem* 2000;275:11993-12002.
13) Nakamura I, Pilkington MF, Lakkakorpi PT et al. Role of alpha(v)beta(3) integrin in osteoclast migration and formation of the sealing zone. *J Cell Sci* 1999;112 (Pt 22):3985-3993.
14) Duong LT, Lakkakorpi PT, Nakamura I, Machwate M, Nagy RM, Rodan GA. PYK2 in osteoclasts is an adhesion kinase, localized in the sealing zone, activated by ligation of a_vb_3 integrin, and phosphorylated by Src kinase. *J Clin Invest* 1998;102:881-892.
15) Wang Q, Xie Y, Du QS et al. Regulation of the formation of osteoclastic actin rings by proline-rich tyrosine kinase 2 interacting with gelsolin. *J Cell Biol* 2003;160:565-575.
16) Hiroi-Furuya E, Kameda T, Hiura K et al. Etidronate (EHDP) inhibits osteoclastic-bone resorption, promotes apoptosis and disrupts actin rings in isolate-mature osteoclasts. *Calcif Tissue Int* 1999;64:219-223.
17) Lakkakorpi PT, Nakamura I, Young M, Lipfert L, Rodan GA, Duong LT. Abnormal localization and hyperclustering of (alpha)(V)(beta)(3) integrins and associated proteins in Src-deficient or tyrphostin A9-treated osteoclasts. *J Cell Sci* 2001;114:149-160.
18) Hurst IR, Zuo J, Jiang J, Holliday LS. Actin-related protein 2/3 complex is required for actin ring formation. *J Bone Miner Res* 2004;19:499-506.
19) Ma T, Samanna V, Chellaiah MA. Dramatic inhibition of osteoclast sealing ring formation

and bone resorption in vitro by a WASP-peptide containing pTyr294 amino acid. *J Mol Signal* 2008;3:4.

20) Chellaiah MA, Moorer MC, Majumdar S et al. L-Plastin deficiency produces increased trabecular bone due to attenuation of sealing ring formation and osteoclast dysfunction. *Bone Res* 2020;8:3.
21) Lakkakorpi P, Tuukkanen J, Hentunen T, Jarvelin K, Vaananen K. Organization of osteoclast microfilaments during the attachment to bone surface *in vitro*. *J Bone Miner Res* 1989;4:817-825.
22) Lakkakorpi PT, Vaananen HK. Kinetics of the osteoclast cytoskeleton during the resorption cycle *in vitro*. *J Bone Miner Res* 1991;6:817-826.
23) Teti A, Marchisio PC, Zambonin-Zallone A. Clear zone in osteoclast function: role of podosomes in regulation of bone-resorbing activity (Review). *Amer J Physiol* 1991;261:C1-C7.
24) Jurdic P, Saltel F, Chabadel A, Destaing O. Podosome and sealing zone: specificity of the osteoclast model. *Eur J Cell Biol* 2006;85:195-202.
25) Saltel F, Destaing O, Bard F, Eichert D, Jurdic P. Apatite-mediated actin dynamics in resorbing osteoclasts. *Mol Biol Cell* 2004;15:5231-5241.
26) Taylor ML, Boyde A, Jones SJ. The effect of fluoride on the patterns of adherence of osteoclasts cultured on and resorbing dentine: a 3-D assessment of vinculin-labelled cells using confocal optical microscopy. *Anat Embryol (Berl)* 1989;180:427-435.
27) McHugh KP, Hodivala-Dilke K, Zheng M-H et al. Mice lacking b3 integrins are osteosclerotic because of dysfunctional osteoclasts. *J Clin Invest* 2000;105:433-440.
28) Feng X, Novack DV, Faccio R et al. A Glanzmann's mutation in beta 3 integrin specifically impairs osteoclast function. *J Clin Invest* 2001;107:1137-1144.
29) Murakami H, Takahashi N, Sasaki T et al. A possible mechanism of the specific action of bisphosphonates on osteoclasts: Tiludronate preferentially affects polarized osteoclasts having ruffled borders. *Bone* 1995;17:137-144.
30) Zhang D, Udagawa N, Nakamura I et al. The small GTP-binding protein, rho p21, is involved in bone resorption by regulating cytoskeletal organization in osteoclasts. *Journal of Cell Science* 1995;108:2285-2292.
31) Duong LT, Rodan GA. Integrin-mediated signaling in the regulation of osteoclast adhesion and activation. *Front Biosci* 1998;3:d757-d768.
32) Izawa T, Zou W, Chappel JC, Ashley JW, Feng X, Teitelbaum SL. c-Src links a RANK/alphavbeta3 integrin complex to the osteoclast cytoskeleton. *Mol Cell Biol* 2012;32:2943-2953.
33) Faccio R, Teitelbaum SL, Fujikawa K et al. Vav3 regulates osteoclast function and bone mass. *Nat Med* 2005;11:284-290.
34) Chellaiah MA, Schaller MD. Activation of Src kinase by protein-tyrosine phosphatase-PEST in osteoclasts: comparative analysis of the effects of bisphosphonate and protein-tyrosine phosphatase inhibitor on Src activation in vitro. *J Cell Physiol* 2009;220:382-393.
35) Chellaiah MA. Regulation of actin ring formation by rho GTPases in osteoclasts. *J Biol Chem* 2005;280:32930-32943.
36) Gupta A, Lee BS, Khadeer MA et al. Leupaxin is a critical adaptor protein in the adhesion zone of the osteoclast. *J Bone Miner Res* 2003;18:669-685.
37) Miyazaki T, Tanaka S, Sanjay A, Baron R. The role of c-Src kinase in the regulation of osteoclast function. *Mod Rheumatol* 2006;16:68-74.
38) Duong LT, Lakkakorpi PT, Nakamura I, Machwate M, Nagy RM, Rodan GA. PYK2 in osteoclasts is an adhesion kinase, localized in the sealing zone, activated by ligation of alpha(v)beta3 integrin, and phosphorylated by src kinase. *J Clin Invest* 1998;102:881-892.
39) Chellaiah MA, Biswas RS, Yuen D, Alvarez UM, Hruska KA. Phosphatidylinositol 3,4,5-

trisphosphate directs association of Src homology 2-containing signaling proteins with gelsolin. *J Biol Chem* 2001;276:47434-47444.
40) Baron R. Molecular mechanisms of bone resorption. An update. *Acta Orthop Scand Suppl* 1995;266:66-70.
41) Teitelbaum SL. The osteoclast and its unique cytoskeleton. *Ann N Y Acad Sci* 2011;1240:14-17.
42) Chellaiah MA, Kuppuswamy D, Lasky L, Linder S. Phosphorylation of a Wiscott-Aldrich syndrome protein-associated signal complex is critical in osteoclast bone resorption. *J Biol Chem* 2007;282:10104-10116.
43) Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-*src* proto-oncogene leads to osteopetrosis in mice. *Cell* 1991;64:693-702.
44) Sanjay A, Houghton A, Neff L et al. Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha(v)beta(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. *J Cell Biol* 2001;152:181-195.
45) Ory S, Munari-Silem Y, Fort P, Jurdic P. Rho and Rac exert antagonistic functions on spreading of macrophage-derived multinucleated cells and are not required for actin fiber formation. *J Cell Sci* 2000;113 (Pt 7):1177-1188.
46) Chellaiah MA. Regulation of podosomes by integrin alphavbeta3 and Rho GTPase-facilitated phosphoinositide signaling. *Eur J Cell Biol* 2006;85:311-317.
47) McMichael BK, Scherer KF, Franklin NC, Lee BS. The RhoGAP activity of myosin IXB is critical for osteoclast podosome patterning, motility, and resorptive capacity. *PLoS ONE* 2014;9:e87402.
48) Teitelbaum SL. Osteoclasts and integrins. *Ann N Y Acad Sci* 2006;1068:95-99.
49) Novack DV, Faccio R. Osteoclast motility: putting the brakes on bone resorption. *Ageing Res Rev* 2011;10:54-61.
50) Calle Y, Jones GE, Jagger C et al. WASp deficiency in mice results in failure to form osteoclast sealing zones and defects in bone resorption. *Blood* 2004.
51) Lee J, Park C, Kim HJ et al. Stimulation of osteoclast migration and bone resorption by C-C chemokine ligands 19 and 21. *Exp Mol Med* 2017;49:e358.
52) Tehrani S, Faccio R, Chandrasekar I, Ross FP, Cooper JA. Cortactin has an essential and specific role in osteoclast actin assembly. *Mol Biol Cell* 2006;17:2882-2895.
53) Iwatake M, Nishishita K, Okamoto K, Tsukuba T. The Rho-specific guanine nucleotide exchange factor Plekhg5 modulates cell polarity, adhesion, migration, and podosome organization in macrophages and osteoclasts. *Exp Cell Res* 2017;359:415-430.
54) Rogers MJ. New insights into the molecular mechanisms of action of bisphosphonates. *Curr Pharm Des* 2003;9:2643-2658.
55) Destaing O, Saltel F, Geminard J-C, Jurdic P, Bard FA. Podosomes display actin turn over and dynamic self organization in osteoclasts expressing actin-green fluorescent protein. Actin-GFP in osteoclasts. Molecular Biology of the Cell 14[2003], 407-416. 2003.
56) Gil-Henn H, Destaing O, Sims NA et al. Defective microtubule-dependent podosome organization in osteoclasts leads to increased bone density in Pyk2(-/-) mice. *J Cell Biol* 2007;178:1053-1064.
57) Faccio R, Takeshita S, Colaianni G et al. M-CSF regulates the cytoskeleton via recruitment of a multimeric signaling complex to c-FMS Y559/697/721. *J Biol Chem* 2007.
58) Croke M, Ross FP, Korhonen M, Williams DA, Zou W, Teitelbaum SL. Rac deletion in osteoclasts causes severe osteopetrosis. *J Cell Sci* 2011;124:3811-3821.
59) Goldberg SR, Georgiou J, Glogauer M, Grynpas MD. A 3D scanning confocal imaging method measures pit volume and captures the role of Rac in osteoclast function. *Bone* 2012;51:145-152.

60) Rifas L. Bone and cytokines: Beyond IL-1, IL-6 and TNF-a. *J Bone Miner Res*. In press.
61) Nakamura I, Kadono Y, Takayanagi H et al. IL-1 regulates cytoskeletal organization in osteoclasts via TNF receptor-associated factor 6/c-Src complex. *J Immunol* 2002;168:5103-5109.
62) Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. IL-1 mediates TNF-induced osteoclastogenesis. *J Clin Invest* 2005;115:282-290.
63) Kamagata Y, Miyasaka N, Inoue H, Hashimoto J, Iida M. [Study of cytokine production in inflamed human gingival tissues in periodontitis. Interleukin-1 (IL-1 alpha, beta) and tumor necrosis factor (TNF alpha)]. *Nihon Shishubyo Gakkai Kaishi* 1989;31:843-848.
64) Pfeilschifter J, Chenu C, Bird A, Mundy GR, Roodman GD. Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclastlike cells in vitro. *J Bone Miner Res* 1989;4:113-118.
65) Suda T, Kobayashi K, Jimi E, Udagawa N, Takahashi N. The molecular basis of osteoclast differentiation and activation. *Novartis Found Symp* 2001;232:235-247.
66) Deo V, Bhongade ML. Pathogenesis of periodontitis: role of cytokines in host response. *Dent Today* 2010;29:60-66.
67) Feng W, Guo J, Li M. RANKL-independent modulation of osteoclastogenesis. *J Oral Biosci* 2019;61:16-21.
68) Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A. Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. *J Biol Chem* 2000;275:4858-4864.
69) Kobayashi K, Takahashi N, Jimi E et al. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *J Exp Med* 2000;191:275-286.
70) Fuller K, Murphy C, Kirstein B, Fox SW, Chambers TJ. TNFalpha potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. *Endocrinology* 2002;143:1108-1118.
71) Luxenburg C, Addadi L, Geiger B. The molecular dynamics of osteoclast adhesions. *Eur J Cell Biol* 2006;85:203-211.
72) Delanote V, Vandekerckhove J, Gettemans J. Plastins: versatile modulators of actin organization in (patho)physiological cellular processes. *Acta Pharmacol Sin* 2005;26:769-779.
73) Foran E, McWilliam P, Kelleher D, Croke DT, Long A. The leukocyte protein L-plastin induces proliferation, invasion and loss of E-cadherin expression in colon cancer cells. *Int J Cancer* 2006;118:2098-2104.
74) Janji B, Vallar L, Al TZ et al. The actin filament cross-linker L-plastin confers resistance to TNF-alpha in MCF-7 breast cancer cells in a phosphorylation-dependent manner. *J Cell Mol Med* 2010;14:1264-1275.
75) de Arruda MV, Watson S, Lin CS, Leavitt J, Matsudaira P. Fimbrin is a homologue of the cytoplasmic phosphoprotein plastin and has domains homologous with calmodulin and actin gelation proteins. *J Cell Biol* 1990;111:1069-1079.
76) Babb SG, Matsudaira P, Sato M, Correia I, Lim S-S. Fimbrin in podosomes of monocyte-derived osteoclasts. *Cell Motil Cytosk* 1997;37:308-325.
77) Hanein D, Volkmann N, Goldsmith S et al. An atomic model of fimbrin binding to F-actin and its implications for filament crosslinking and regulation. *Nat Struct Biol* 1998;5:787-792.
78) Namba Y, Ito M, Zu Y, Shigesada K, Maruyama K. Human T cell L-plastin bundles actin filaments in a calcium-dependent manner. *J Biochem (Tokyo)* 1992;112:503-507.
79) Volkmann N, DeRosier D, Matsudaira P, Hanein D. An atomic model of actin filaments cross-linked by fimbrin and its implications for bundle assembly and function. *J Cell Biol* 2001;153:947-956.
80) Janji B, Giganti A, De C, V et al. Phosphorylation on Ser5 increases the F-actin-

binding activity of L-plastin and promotes its targeting to sites of actin assembly in cells. *J Cell Sci* 2006;119:1947-1960.

81) Feng X. Regulatory roles and molecular signaling of TNF family members in osteoclasts. *Gene* 2005;350:1-13.
82) Ma T, Sadashivaiah K, Chellaiah MA. Regulation of sealing ring formation by L-plastin and cortactin in osteoclasts. *J Biol Chem* 2010;285:29911-29924.
83) Chellaiah MA, Ma T, Majumdar S. L-plastin phosphorylation regulates the early phase of sealing ring formation by actin bundling process in mouse osteoclasts. *Exp Cell Res* 2018;372:73-82.
84) Chellaiah MA, Majumdar S, Aljohani H. Peptidomimetic inhibitors of L-plastin reduce the resorptive activity of osteoclast but not the bone forming activity of osteoblasts in vitro. *PLoS ONE* 2018;13:e0204209.
85) Majumdar S, Wadajkar AS, Aljohani H, Reynolds MA, Kim AJ, Chellaiah M. Engineering of L-Plastin Peptide-Loaded Biodegradable Nanoparticles for Sustained Delivery and Suppression of Osteoclast Function In Vitro. *Int J Cell Biol* 2019;2019:6943986.
86) Si M, Goodluck H, Zeng C et al. LRRK1 regulation of actin assembly in osteoclasts involves serine 5 phosphorylation of L-plastin. *J Cell Biochem* 2018;119:10351-10357.

