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PYK2 AND FAK IN OSTEOCLASTS

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1. ABSTRACT

Integrin alpha$_v$beta$_3$ plays a pivotal role in osteoclastic bone resoprtion by mediating both osteoclast attachments on bone matrix and cytoskeleton reorganization. The focal adhesion kinase (FAK) family kinases including FAK and proline-rich tyrosine kinase 2 (PYK2) are major tyrosine kinases activated by integrin engagement. In osteoclasts, although FAK is expressed, PYK2 appears to be the predominant mediator of integrin alpha$_v$beta$_3$ signaling events that influence osteoclast physiology and pathology. Recent progress in the understanding of the role of PYK2/FAK kinases in the regulation of osteoclastic actin cytoskeletal organization, cell migration, and bone resorption will be discussed in this review.

2. INTRODUCTION

Osteoclasts are our body's principal bone resorbing cells that not only play a critical role in skeleton development and maintenance but are also implicated in the pathogenesis of various bone diseases including postmenopausal osteoporosis (1-3). Osteoclasts are multinucleated giant cells that differentiate from cells of hematopoietic origin (1;4;5). Hematopoietic stem cells give rise to circulating mononuclear cells which are attracted to attach to bone-resorbing sites to differentiate into osteoclasts in response to two key osteoclastogenic factors M-CSF and RANKL (6-11). Mature osteoclasts need to remain attached on bone matrix to degrade bone. Thus, both osteoclast differentiation and function depend on a physical interaction between the multinucleated giant cell and bone matrix.

Integrins are heterodimeric transmembrane glycoproteins that not only mediate cell-extracellular and cell-cell interactions but also transduce intracellular signaling important for cell function (12;13). Integrins play critical roles in regulating distinct biological processes in a variety of cell types including osteoclasts (12-15). Many integrins are found on mature osteoclasts (14;15). However, integrin alpha$_v$beta$_3$ has been shown to play a predominant role in bone resorption by mediating not only the osteoclast attachment on the bone matrix but also cytoskeleton reorganization, which is important for osteoclatic bone resorprtion (14;15). Numerous studies in the past several years have established that cytoplasmic tyrosione kinases FAK and PYK2 are two important players involved in integrin alpha$_v$beta$_3$-mediated signaling pathways in osteoclast function.

Since several recent reviews have provided excellent general reviews on the role of integrins, particularly integrin alpha$_v$beta$_3$, in osteoclast function (7;14-16), in the current review we will focus on discussing the current understanding of roles of FAK and PYK2 in integrins alpha$_v$beta$_3$-mediated signaling in osteoclasts. Moreover, as an introduction to FAK and PYK2, Chapter one in this review series provides a comprehensive review on FAK and PYK2. Thus, for a general review on FAK and PYK2, we would like to refer our readers to Chapter one in this review series.

3. DISCUSSION

3.1. Integrins and Osteoclasts

3.1.1. Mechanism of Osteoclastic Bone Resorption

Osteoclastic bone resorption is a complicated process involving multiple steps (7;17;18). To better understand the role of FAK and PYK2 in osteoclastic bone resorption, we will briefly review the mechanism of

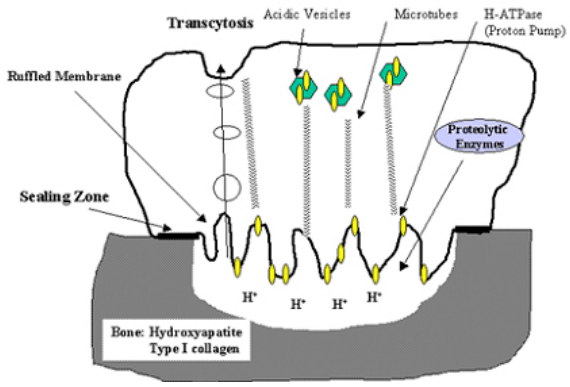

Figure 1. Mechanism of Osteoclastic Bone Resorption. Several structural features of osteoclasts are important for bone resorption. To resorb bone, osteoclasts tightly attach onto bone matrix through the "sealing zone", which seals the resorption site from its surroundings. The second feature is the formation of ruffled membrane that is rich in proton pumps (H-ATPase). Proton pumps transport protons into the resorption site to dissolve the inorganic bone matrix (hydroxyapatite). In addition, the ruffled membrane also transport proteolytic enzymes that degrade the organic bone matrix (collagen I). The ruffled membrane is formed by fusion of intracellular acidic vesicles via microtubes. Degraded products are removed by transcytosis.

osteoclastic bone resorption with a focus on several osteoclast functional structures involving FAK and PYK2. Figure 1 illustrates a typical osteoclast residing on bone and highlights several morphological features that are essential for bone resorption. First, the interaction between the giant bone-resorbing cell and bone is mediated by a distinct matrix attachment often referred to as "sealing zone" (19). The sealing zone, organized as a ring, enables the cell to tightly attach onto bone to seal the resorption site from its surroundings. Moreover, the sealing zone contains many punctate plasma membrane protrusions known as podosomes (20;21), which are structurally related to focal adhesions. Podosomes contain not only adhesion molecules such as integrins that mediate the osteoclast attachment on bone matrix but also cytoskeleton proteins and intracellular signaling proteins on their intracellular side (22-26), suggesting that podosomes play a role in linking the matrix recognition to cytoskeleton formation/reorganization. In particular, the sealing zone is rich in actin filaments polarized perpendicularly to bone surface (27). Similar to the sealing zone, the actin cytoskeleton is also organized as a ring, thus being referred to as "actin ring". Podosomes are involved in organizing the actin cytoskeleton, which plays an important role in mediating osteoclastic bone resorption and migration (7).

Another important feature of osteoclasts is the presence of the ruffled membrane (Figure 1). The ruffled membrane is rich in H^+-ATPase (proton pump) that transports protons into the sealed resorption site to maintain a low pH environment essential for dissolution of inorganic components of bone (28). The ruffled membrane also transports proteolytic enzymes that degrade the organic bone matrix after dissolution of the mineral component (29-34). The ruffled membrane is formed by fusion of intracellular acidic vesicles with the region of plasma membrane facing the bone (28;35;36). Transport of these acidic vesicles depend on microtubes. The last step of a bone resorption cycle is the removal of degraded products, which is mediated by transcytosis (Figure 1). Microtubule is also implicated in transcytosis (37;38).

3.1.2. Integrin $alpha_v beta_3$ is Involved in Regulating Osteoclastic Bone Resorption

As discussed above, osteoclastic bone resorption requires a tight physical interaction between the cell and bone matrix. The identification and characterization of adhesion molecules involved in mediating the interaction was a major focus of bone biology research in the past decade or so. As a result, it has now been established that integrins play a central role in this process (14;15).

Mature osteoclasts express a variety of integrins, including integrin $alpha_v beta_3$, $alpha_2 beta_1$, $alpha_v beta_1$, $alpha_M beta_2$ (39-42). Among these integrins, integrin $alpha_v beta_3$ was shown to play a predominant role in osteoclast attachment and bone resorption. Initial evidence supporting this notion came from an *in vitro* study showing that a monoclonal antibody against an antigen on osteoclasts inhibits bone resortiopn (43) and the antigen was later identified as integrin $alpha_v beta_3$ (44). Consistently, an independent study showed that LM609, a blocking antibody recognizing avian integrin $alpha_v beta_3$, not only blocks the avian osteoclast attachment onto bone but also bone resorption (45). Subsequently, integrin $alpha_v beta_3$ was shown to mediate osteoclast attachment by recognizing the RGD sequence present in various bone matrix proteins such as osteopontin, vitronectin, and bone sailoprotein (46-49).

In line with the *in vitro* data, integrin $beta_3$ knockout mice exhibited an osteoscloretic phenotype due to a functional defect in osteoclasts, confirming that intgein $alpha_v beta_3$ is important for osteoclast function *in vivo* (50). Integrin $beta_3^{-/-}$ osteoclasts failed to form both the actin ring and the ruffled membrane (50), indicating that $alpha_v beta_3$ plays a critical role in the actin filament and microtubule assembly. More significantly, *in vitro* rescue study with the integrin $beta_3^{-/-}$ osteoclasts further revealed that integrin $alpha_v beta_3$ not only serves as anchoring molecules mediating interaction between osteoclasts and bone, but more importantly it also transmits intracellular signaling involved in bone resorption (51). In particular, this same study showed that S^{752} in the integrin $beta_3$ cytoplasmic domain is an essential residue for integrin $alpha_v beta_3$-mediated signaling in bone resorption (51).

Thus, both *in vitro* and *in vivo* data support an important role for integrin $alpha_v beta_3$-mediated signaling in bone resorption. Furthermore, the studies in the last decade have also implicated both FAK and its related cousin PYK2 as two signaling molecules in this process. Below we will discuss the current understanding of role of FAK and PYK2 in intgerin $alpha_v beta_3$-mediated signaling pathways involved in bone resorption.

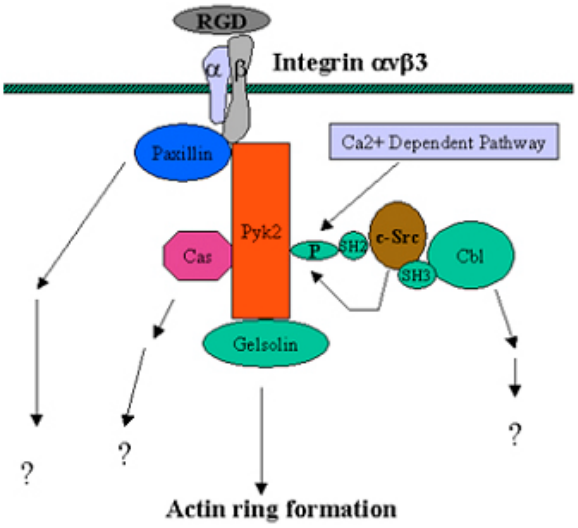

Figure 2. Role of PYK2 in Integrin αvβ3-mediated Intracellular Siganling in Osteoclasts. In response to integrin αvβ3 activation, PYK2 is recruited to the podosomes through direct interactions with the β3 integrin cytoplasmic tails. In the integrin αvβ3-induced signaling complex, PYK2 interacts with paxillin and Cas. However, the functional roles of these interactions have not been clearly revealed. In addition, integrin αvβ3 activation also induces PYK2 phosphorylation by c-Src and/or a Ca2+-dependent pathway. Phosphorylated PYK2 interacts with other signaling molecules such as c-Src to form signaling complex together with c-Cbl, which was proposed to play a role in osteoclast function. But, the role of this complex in osteoclast function has not been confirmed. Most significantly, recent studies indicate that PYK2 interacts and phosphorylates gelsolin to mediate actin ring formation.

3.2. PYK2 in Osteoclasts

3.2.1. Functional Expression of PYK2 in Osteoclasts

PYK2, a FAK-related cytoplasmic kinase, appears to be a major cell adhesion activated tyrosine kinase in osteoclasts (22). In line with this initial report, numerous other studies also found that PYK2 is expressed at higher levels than FAK in osteoclasts (52-54). In addition to its abundant expression in osteoclasts, PYK2 is tyrosine phosphorylated in response to ligation of integrin $alpha_v beta_3$ by RGD-containing peptide or proteins. PYK2 is localized to podosomes and ring-line structures in osteoclasts (22), suggesting that PYK2 may play a functional role in osteoclast function. Importantly, inhibition of PYK2 by antisense experiments demonstrated that PYK2 plays an important role in integrin $alpha_v beta_3$-mediated cytoskeletal organization and formation of the sealing zone essential for osteoclast function (55).

3.2.2. PYK2 in Integrin $alpha_v beta_3$-mediated Signaling in Osteoclasts

The initial demonstration of functional expression of PYK2 by Duong and coworkers represents a beginning of extensive investigations of PYK2-mediated signaling pathways involved in osteoclast function (22). It has now been established that PYK2 plays a central role in linking the integrin $alpha_v beta_3$ activation to the formation of podosomes and actin rings that are critical for osteoclast function. Figure 2 summarizes the current understanding of the role of PYK2 in integrin $alpha_v beta_3$-mediated signaling in osteoclasts.

Upon the adhesion of osteoclasts on bone matrix, which is primarily mediated by integrin $alpha_v beta_3$, PYK2 translocates into the Triton X-100 insoluble cytoskeletal fraction and localizes to posdosomes in osteoclasts (22) (Figure 2). A recent study showed that PYK2 directly interacts with the integrin $beta_3$ cytoplasmic tail in *in vitro* binding assays (23), suggesting that the recruitment of PYK2 to podosomes is mediated through a direct interaction between PYK2 and the integrin $beta_3$ cytoplasmic tail. Consistently, the $S^{752}P$ mutation in integrin $beta_3$ cytoplasmic tail, which was previously shown to abrogate the integrin $beta_3$ function in bone resorption (51), also disrupts the interaction between PYK2 and the integrin $beta_3$ cytoplasmic tail (23).

The integrin $alpha_v beta_3$-dependent adhesion of osteoclasts on bone matrix not only induces localization of PYK2 to podosomes but also causes PYK2 phosphorylation (22;23). PYK2 phosphorylation correlates with the sealing zone formation and bone resorption (22), suggesting that PYK2 phosphorylation may be critical for its function in bone resorption. In further support of this notion, it was shown that treatment of osteoclasts with calcitonin, an inhibitor of osteoclastic bone resorption, resulted in dephosphorylation of PYK2 (52). Since PYK2 phosphorylation is required for its binding to c-Src via SH2 domain (22;56), PYK2 phosphorylation may be involved in regulating bone resorption through c-Src (Figure 2), an important kinase for osteoclastic bone resoprtion (57). However, how PYK2 is phosphorylated in response to integrin $alpha_v beta_3$ ligation remains controversial. One report showed that PYK2 is phosphorylated by c-Src in osteoclasts (22). In this report, it was not only shown that PYK2 can be phosphorylated by recombinant c-Src in *in vitro* kinase assays, more importantly but also that PYK2 phosphorylation is significantly reduced in osteoclasts derived from c-Src knockout mice (22). In contrast, another group showed that the phosphorylation of PYK2 results from a Ca^{2+}-dependent pathway (56). Notably, this study specifically indicated that PYK2 phosphorylation is not reduced in osteoclasts derived from c-$Src^{-/-}$ mice (56). Given the involvement of PYK2 phosphorylation in integrin $alpha_v beta_3$-mediated bone resorption, more studies are needed to define the precise mechanism by which PYK2 is phosphorylated in response to integrin $alpha_v beta_3$ ligation.

While PYK2 phosphorylation plays a role in mediating the interaction of PYK2 with other signaling proteins present in the integrin $alpha_v beta_3$-dependent signaling complex, PYK2 kinase activity is likely responsible for transmitting downstream signals by phosphorylating downstream proteins (Figure 2). Supporting this possibility, inhibition of bone resorption by calcitonin or echistatin is associated with a reduction in

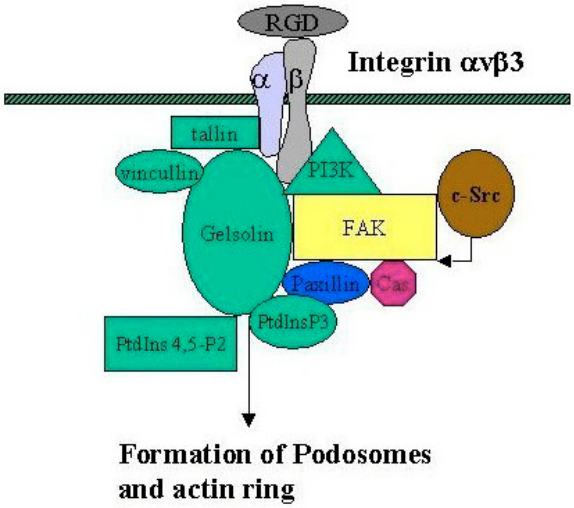

Figure 3. Role of FAK in Integrin αvβ3-mediated Intracellular Siganling in Osteoclasts. Primarily based on studies with avian osteoclasts, FAK is implicated in the formation of a signaling complex consisting of many cytoskeletal and signaling molecules including talin, vincullin, paxillin, PI3K, gelsolin, Cas, c-Src, PtdIns 4,5-P2, PtdInsP3 and FAK. FAK is phosphorylated by c-Src. However, the precise downstream targets of FAK is unknown.

PYK2 kinase activity (22). Moreover, treatment of osteoclasts with cytochalasin D inhibits PYK2 kinase activity, suggesting that cytoskeleton is involved in regulating PYK2 kinase activity. So, how is PYK2 kinase activity involved in regulating bone resorption? It has been shown that PYK2 can phosphorylate several proteins present in podosomes, including $p130^{Cas}$ (55), paxillin (58;59) and gelsolin (54). Given that paxillin is cytoskeletal protein, its phosphorylation by PYK2 may be involved in PYK2-dependent cytoskeletal organization in osteoclasts. However, despite the interaction of $p130^{Cas}$ with PYK2 and phosphorylation of $p130^{Cas}$ by PYK2, the precise role of $p130^{Cas}$ in integrin $alpha_v beta_3$-mediated osteoclastic bone resorption remains poorly characterized.

Significantly, it has been revealed that phosphorylation of gelsolin by PYK2 plays a pivotal role in mediating actin ring formation in osteoclasts (54) (Figure 2). In this study, it was showed that PYK2 directly interacts with gelsolin and that the interaction between PYK2 and gelsolin is mediated by the focal-adhesion-targeting domain in PYK2 and a LD motif in gelsolin's C-terminus (54). In addition, PYK2 phosphorylates gelsolins at tyrosine residues. The phosphorylated gelsolins exhibit lower affinity for actin monomers but higher affinity for phosphatidylinositol lipids, leading to uncap actin filaments at the barbed ends, increase actin polymerization at the cell periphery and the formation of actin ring (54).

Notably, it was also shown that PYK2 plays a role in recruiting c-Cbl in a signaling complex consisting of PYK2, c-Src and c-Cbl in response to integrin $alpha_v beta_3$ activation (56) (Figure 2). This signaling complex leads to phosphorylation of c-Cbl (56). However, the precise role of c-Cbl in osteoclast function has still been definitively characterized. In particular, c-Cbl knockout mice show no bone abnormality (60).

3.3. FAK in Osoteclasts

3.3.1. Functional Expression of FAK in Osoteclasts

A potential role of FAK in osteoclastic bone resorption is suggested by studies demonstrating that FAK is expressed in osteoclasts. Using immunofluoresent methods, Berry and coworkers showed for the first time that FAK is expressed abundantly in both human and avian osteoclasts (61). Moreover, FAK expression in osteoclasts is inhibited by calcitonin (61), an inhibitor of osteoclast function (62;63), suggesting that FAK may play a role in regulating osteoclastic bone resorption. Consistently, mouse osteocalsts also express FAK and FAK expression is primarily localized at the periphery of mouse osteoclasts (64). More importantly, this study demonstrated that inhibition of FAK expression by an antisense strategy decreased the capacity of osteoclasts to resorb bone (64), providing a direct *in vitro* data supporting a functional role of FAK in bone resorption. Despite these *in vitro* data, the role of FAK in osteoclastic bone resorption *in vivo* has not been confirmed, primarily because FAK knockout mice are embryonic lethal.

Phosphorylation of FAK may be important for its role in bone resorption. One study showed that suppression of FAK phosphorylation not only changed the localization of FAK but also blocked actin-ring formation (64), suggesting a positive role of FAK phosphorylation in osteoclastic bone resorption (64). In contrast, a recent report indicates that calcitonin induces phosphorylation of FAK in osteoclasts (52). The calcitonin-induced FAK phosphorylation is associated with disruption of the actin ring and the dissociation of FAK at the periphery of osteoclasts (52), implying that FAK phosphorylation may be negatively involved in bone resorption.

3.3.2. FAK in Integrin $alpha_v beta_3$-mediated Signaling in Osteoclasts

With the demonstration of functional expression of FAK in osteoclasts, the precise role of FAK in osteoclasts has just begun to be unraveled. The available data support that FAK is primarily implicated in integrin $alpha_v beta_3$-mediated signaling in osteoclasts. In consistency with its involvement in integrin $alpha_v beta_3$-mediated signaling, FAK is present in podosomes and regulates actin ring formation (52;64). Mainly based on studies with avian osteoclasts, Chellaiah and coworkers has proposed a model describing integrin $alpha_v beta_3$-initiated signaling in osteoclasts (65). Central to this model is the involvement of gelsolin in integrin $alpha_v beta_3$-initiated signaling in osteoclasts (Figure 3). Gelsolin is an actin-binding protein that controls the length of the actin filament, which is important for various cell functions such as cell motility (66-68). Ligation of integrin $alpha_v beta_3$ with RGD-containing proteins such as osteopontin activates gelsolin-associated PI3K, resulting in enhanced association of gelsolin with phosphoinositotides such as PtdIns 4,5-P2 and PtdIns P3 (69) (Figure 3). The association of gelsolin with phosphoinositotides induces the release of gelsolin

from the actin filament end, leading to the actin ring formation and reorganization (26).

FAK represents one of components present in the integrin alpha$_v$beta$_3$-activated signaling complex (65) (Figure 3). Although the precise role of FAK in the integrin alpha$_v$beta$_3$-activated signaling FAK is not known, FAK may participate in this process in part by stimulating the formation of the signaling complex. In support of this notion, FAK is phosphorylated in response to integrin alpha$_v$beta$_3$ activation and phosphorylated FAK associate with other signaling molecules such as c-Src, PI3K, paxillin and p130cas (70-74), all of whom were shown to be present in the signaling complex (65). Phosphorylation of FAK in osteoclasts results from the action of c-Src (75). Consistently, kinase activity of c-Src is enhanced by integrin alpha$_v$beta$_3$ ligation in osteoclasts (76). Nonetheless, the downstream target(s) of FAK in the signaling process leading to actin ring formation has not been elucidated (Figure 3). As we have discussed above, PYK2, a FAK-related kinase, is implicated in integrin alpha$_v$beta$_3$-mediated signaling by phosphorylating gelsolin (54). But that study also showed that FAK is not involved in phosphorylating gelsolin (54).

4. SUMMARY AND PERSPECTIVE

Research data accumulated in last several years have pointed to a functional role for both FAK and PYK2 in osteoclastic bone resorption. Both FAK and PYK2 were shown to be not only present in osteoclasts but also implicated in integrin alpha$_v$beta$_3$-mediated bone resorption. Moreover, the roles of FAK and PYK2 in the integrin alpha$_v$beta$_3$-mediated signaling have begun to be elucidated. Undoubtedly, these findings have significantly advanced our understanding of the mechanism of osteoclastic bone resorption. On the other hand, as we have discussed above, the data from different research groups have also created several considerable controversies regarding the role of FAK and PYK2 in osteoclasts. While some discrepancies may result from the different model systems used or from the experiments performed under different conditions, other may simply represent true biological differences that we have not appreciated. For instance, a controversy exists regarding whether FAK or PYK2 is involved in integrin alpha$_v$beta$_3$-mediated signaling in podosomes in osteoclasts. Interestingly, a recent study showed that a majority of podosomes exclusively contains PYK2 while a few podosomes have only FAK (52), suggesting that both PYK2 and FAK may be involved in osteoclast function. Thus, future studies aimed at addressing these controversies will not only definitively identify the precise roles of FAK and PYK2 in osteoclasts but may also elucidate novel mechanisms implied by some of these controversies.

Furthermore, most studies on FAK and PYK2 have focused on their role in integrin alpha$_v$beta$_3$-mediated signaling involved in bone resorption. However, emerging evidence support that FAK and PYK2 are also involved in signaling pathways activated by other factors known to regulate osteoclast function. Treatment of osteoclasts with calcitonin induces PYK2 dephosphorylation but stimulates FAK phosphorylation (52). M-CSF, a critical factor for osteoclast formation and function, induces the interaction of PLC-gamma with PYK2 (77), suggesting that PYK2 may act as a linker mediating the cross talk between integrin alpha$_v$beta$_3$-mediated signaling and that initiated by M-CSF. In addition, VEGF, an angiogenic factor that was recently shown to regulate osteoclast function (78), is also capable of inducing phosphorylation of FAK in an osteoclast precursor cell line (79). These data suggest that FAK and PYK2 may also be involved in other signaling pathways in osteoclasts and that they may play an important role in mediating the potential crosstalk between integrin alpha$_v$beta$_3$-initaied signaling and those activated by other factors. Future investigations on the roles of FAK and PYK2 in signaling pathways activated by other factors will provide more insights into the mechanism of osteoclastic bone resorption.

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6. REFERENCES

1. Ross, F. P. & S. L. Teitelbaum: Osteoclast Biology. In: Osteoporosis. Eds: Marcus, R., Feldman, D. & Kelsey, J., Academic Press, San Diego, CA, 73-106 (2001)
2. Pacifici, R.: Postmenopausal Osteoporosis: How the Hormonal Changes of Menopausal Cause Bone Loss. In: Osteoporosis. Eds: Marcus, R., Feldman, D. & Kelsey, J., Academic Press, San Diego, CA, 727-744 (1996)
3. Manolagas, S. C.: Cellular and molecular mechanisms of osteoporosis. [Review] [69 refs]. *Aging* (Milano) 10, 182-190 (1998)
4. Suda, T., N. Takahashi & T. J. Martin: Modulation of osteoclast differentiation. *Endocr. Revs*. 13, 66-80 (1992)
5. Teitelbaum, S. L., M. M. Tondravi & F. P. Ross: Osteoclasts, macrophages, and the molecular mechanisms of bone resorption. *J.Leukocyte Biol*. 61, 381-388 (1997)
6. Suda, T., N. Takahashi, N. Udagawa, E. Jimi, M. T. Gillespie & T. J. Martin: Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr.Revs.* 20, 345-357 (1999)
7. Teitelbaum, S. L.: Bone resorption by osteoclasts. *Science* 289, 1504-1508 (2000)
8. Kong, Y.Y., H. Yoshida, I. Sarosi, H.L. Tan, E. Timms, C. Capparelli, S. Morony, A. J. Oliveira-dos-Santos, G. Van, A. Itie, W. Khoo, A. Wakeham, C. R. Dunstan, D. L. Lacey, T. W. Mak, W. J. Boyle & J. M. Penninger: OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 397, 315-323 (1999)
9. Felix, R., M. G. Cecchini & H. Fleisch: Macrophage colony stimulating factor restores *in vivo* bone resorption in the op/op mouse. *Endocrinol.* 127, 2592-2594 (1990)

10. Kodama, H., A. Yamasaki, M. Nose, S. Niida, Y. Ohgama, M. Abe, M. Kumegawa & T. Suda: Congenital osteoclast deficiency in osteopetrotic (op/op) mice is cured by injections of macrophage colony-stimulating factor. *J.Exp.Med.* 173, 269-272 (1991)
11. Wiktor-Jedrzejczak, W., A. Bartocci, A. W. Jr. Ferrante, A. Ahmed-Ansari, K.W. Sell, J.W. Pollard & E. R. Stanley: Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (op/op) mouse. *Proc.Natl.Acad.Sci.U.S.A*. 87, 4828-4832 (1990)
12. Hynes, R. O.: Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69,11-25 (1992)
13. Schwartz, M. A., M. D. Schaller & M.H. Ginsberg: Integrins: emerging paradigms of signal transduction. *Annual Review of Cell & Developmental Biology* 11, 549-599 (1995)
14. Duong, L., P. Lakkakorpi, I. Nakamura & G. A Rodan: Integrins and signaling in osteoclast function [Review]. *Matrix Biology* 19, 97-105 (2000)
15. Teitelbaum, S. L.: Osteoclasts, integrins, and osteoporosis. [Review] [24 refs]. *Journal of Bone & Mineral Metabolism* 18, 344-349 (2000)
16. Rodan, S. B. & G. A. Rodan: Integrin function in osteoclasts. *J.Endocrinol*. 154 Suppl, S47-S56 (1997)
17. Vaananen, H. K., H. Zhao, M. Mulari & J.M. Halleen: The cell biology of osteoclast function. *J.Cell Sci*. 113, 377-381 (2000)
18. Blair, H .C.: How the osteoclast degrades bone. *BioEssays* 20, 837-846 (1998)
19. Vaananen, H. K. & M. Horton: The osteoclast clear zone is a specialized cell-extracellular matrix adhesion structure. *J.Cell Sci.* 108, 2729-2732 (1995)20. Zambonin-Zallone, A., A. Teti, A. Carano & P. C. Marchisio: The distribution of podosomes in osteoclasts cultured on bone laminae: effect of retinol. *J.Bone Miner.Res*. 3, 517-523 (1988)
21. Marchisio, P. C., D. Cirillo, L. Naldini, M. V. Primavera, A. Teti & A. Zambonin-Zallone: Cell-substratum interaction of cultured avian osteoclasts is mediated by specific adhesion structures. *J.Cell Biol*. 99, 1696-1705 (1984)
22. Duong, L. T., P. T. Lakkakorpi, I. Nakamura, M. Machwate, R. M. Nagy & G. A. Rodan: 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.* 102, 881-892 (1998)
23. Pfaff, M. & P. Jurdic: Podosomes in osteoclast-like cells: structural analysis and cooperative roles of paxillin, proline-rich tyrosine kinase 2 (Pyk2) and integrin alphaVbeta3. *J.Cell Sci.* 114, 2775-2786 (2001)
24. Lakkakorpi, P. T., I. Nakamura, R. M. Nagy, J. T. Parsons, G. A Rodan & L. T. Duong: Stable association of PYK2 and p130(Cas) in osteoclasts and their co-localization in the sealing zone. *J.Biol.Chem.* 274, 4900-4907 (1999)
25. Boyce, B. F., T. Yoneda, C. Lowe, P. Soriano & G. R. Mundy: Requirement of pp60c-src expression for osteoclasts to form ruffled borders and resorb bone in mice. *J.Clin.Invest.* 90, 1622-1627 (1992)
26. Chellaiah, M. & K. Hruska: Osteopontin stimulates gelsolin-associated phosphoinositide levels and phosphatidylinositol triphosphate-hydroxyl kinase. *Mol.Biol.Cell* 7, 743-753 (1996)
27. Holtrop, M. E.: Light and electronmicroscopic structure of osteoclasts. In: Bone. Eds: B. K. Hall, CRC Press, Boca Raton, FL. 1-29 (1991)
28. Blair, H. C., S. L. Teitelbaum, R. Ghiselli & S. Gluck: Osteoclastic bone resorption by a polarized vacuolar proton pump. *Science* 245, 855-857 (1989)
29. Blair, H. C., S. L. Teitelbaum, P.A. Schimke, J. D. Konsek, C. M. Koziol & P.H. Schlessinger: Receptor-mediated uptake of mannose-6-phosphate bearing glycoprotein by isolated chicken osteoclasts. *J.Cell.Physiol.* 137, 476-482 (1988)
30. Baron, R., L. Neff, W. Brown, D. Louvard & P. J. Courtoy: Selective internalization of the apical plasma membrane and rapid redistribution of lysosomal enzymes and mannose-6-phosphate receptors during osteoclst inactivation by calcitonin. *J.Cell Sci.* 97, 439-447 (1990)
31. Blair, H. C., S. L. Teitelbaum, L. E. Grosso, D. L. Lacey, H. -L. Tan, D. W. McCort & J. J. Jeffrey: Extracellular matrix degradation at acid pH: Avian osteoclast acid collagenase isolation and characterization. *Biochem.J.* 290, 873-884 (1993)
32. Gowen, M., F. Lazner, R. Dodds, R. Kapadia, J. Feild, M. Tavaria, I. Bertoncello, F. Drake, S. Zavarselk, I. Tellis, P. Hertzog, C. Debouch & I. Kola: Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. *J.Bone Miner.Res*. 14, 1654-1663 (1999)
33. Hamilton, J. A., S. Lingelbach, N. C. Partridge & T. J. Martin: Regulation of plasminogen activator by bone-resorbing hormones in normal and malignant osteoblasts. *Endocrinol.* 116, 2186-2191 (1985)
34. Meikle, M. C., S. Bond, R. M. Hembry, J. Compston, P. I. Croucher & J. J. Reynolds: Human osteoblasts in culture synthesize collagenase and other matrix metalloproteinases in response to osteotropic hormones and cytokines. *J.Cell Sci.* 103, 1093-1099 (1992)
35. Vaananen, H. K., E. K. Karhukorpi, K. Sundquist, B. Wallmark, I. Roininen, T. Hentunen, J. Tuukkanen & P. Lakkakorpi: Evidence for the presence of a proton pump of the vacuolar H(+)-ATPase type in the ruffled borders of osteoclasts. *J.Cell Biol.* 111, 1305-1311 (1990)
36. Palokangas, H., M. Mulari & H. K. Vaananen: Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts. *J.Cell Sci.* 110, 1767-1780 (1997)
37. Nesbitt, S. A. & M. A. Horton: Trafficking of matrix collagens through bone-resorbing osteoclasts. *Science* 276, 266-269 (1997)
38. Salo, J., P. Lehenkari, M. Mulari, K. Metsikko & H. K. Vaananen: Removal of osteoclast bone resorption products by transcytosis. *Science* 276, 270-273 (1997)
39. Clover, J., R. A. Dodds & M. Gowen: Integrin subunit expression by human osteoblasts and osteoclasts *in situ* and in culture. *J Cell Sci* 92, 267-271 (1992)
40. Hughes, D. E., D. M. Salter, S. Dedhar & R. Simpson: Integrin expression in human bone. *J.Bone Miner.Res.* 93, 527-533 (1993)
41. Nesbitt, S., A. Nesbit, M. Helfrich & M. A. Horton: Biochemical characterization of human osteoclast integrins. Osteoclasts express $alpha_v beta_3$, $alpha_2 beta_1$ and $alpha_v beta_1$ integrins. *J.Cell Biol.* 268, 16737-16745 (1993)
42. Duong, L. T., H. Tanaka, G. Wesolowski & G. A. Rodan: Role of $alpha_M beta_2$ integrin in osteoclast differentiation. *J.Bone Miner.Res*. 8, S378 (1993)

43. Chambers, T. J., K. Fuller, J. A. Darby, J. A. S. Pringle & M. A Horton: Monoclonal antibodies against osteoclasts inhibit bone resorption *in vitro*. *Bone Miner.* 1, 127-135 (1986)
44. Davies, J., J. Warwick, N. Totty, R. Philp, M. Helfrich & M. A. Horton: The osteoclast functional antigen, implicated in the regulation of bone resorption, is biochemically related to the vitronectin receptor. *J.Cell Biol.* 109, 1817-1826 (1989)
45. Miyauchi, A., J. Alvarez, E. M. Greenfield, A. Teti, M. Grano, S. Colucci, A. Zambonin-Zallone, F. P. Ross, S. L. Teitelbaum & D. Cheresh: Recognition of osteopontin and related peptides by an $alpha_v beta_3$ integrin stimulates immediate cell signals in osteoclasts. *J.Biol.Chem.* 266, 20369-20374 (1991)
46. Ross, F. P., J. I. Alvarez, J. Chappel, D. Sander, W. T. Butler, M. C. Farach-Carson, K. A. Mintz, P. G. Robey, S. L. Teitelbaum & D. A. Cheresh: Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osetoclast integrin $alpha_v beta_3$ potentiate bone resorption. *J.Biol.Chem*. 268, 9901-9907 (1993)
47. Flores, M., M. Norgard, D. Heinegard, F. P. Reinholt & G. Andersson: RGD-directed attachment of isolated rat osteoclasts to osteopontin, bone sialoprotein, and fibronectin. *Exp.Cell Res*. 201, 526-530 (1992)
48. Helfrich, M. H., S. A. Nesbitt, E. L. Dorey & M. A. Horton: Rat osteoclasts adhere to a wide range of RGD (Arg-Gly-Asp) peptide-containing proteins, including the bone sialoproteins and fibronectin, via a beta 3 integrin. *J.Bone Miner.Res*. 7, 335-343 (1992)
49. Helfrich, M. H., S. A. Nesbitt, P. T. Lakkakorpi, M. J. Barnes, S. C. Bodary, G. Shankar, W. T. Mason, D. L. Mendrick, H. K. Vaananen & M. A. Horton: $Beta_1$ integrins and osteoclast function: Involvement in collagen recognition and bone resorption. *Bone* 19, 317-328 (1996)
50. McHugh, K. P., K. Hodivala-Dilke, M. H. Zheng, N. Namba, J. Lam, D. Novack, X. Feng, F. P. Ross, R. O. Hynes & S. L. Teitelbaum: Mice lacking beta3 integrins are osteosclerotic due to dysfunctional osteoclasts. *J.Clin.Invest*. 105, 433-440 (2000)
51. Feng, X., D. V. Novack, R. Faccio, D. S. Ory, K. Aya, M. L. Boyer, K. P. McHugh, F. P. Ross & S. L. Teitelbaum: A Glanzmann's mutation in beta 3 integrin specifically impairs osteoclast function. *J.Clin.Invest*. 107, 1137-1144 (2001)
52. Zhang, Z., L. Neff, A. L. Bothwell, R. Baron & W. C. Horne: Calcitonin induces dephosphorylation of Pyk2 and phosphorylation of focal adhesion kinase in osteoclasts. *Bone* 31, 359-365 (2002)
53. Levy J. B., E. DiDomenico, L. Neff, I. Dikic, J. Schlessinger & R. Baron: The FAK-related kinase, Pyk2, is tyrosine phosphorylated in response to integrin stimulation in osteoclasts. *J.Bone Miner.Res.* 12, S109 (1997)
54. Wang, Q., Y. Xie, Q. S. Du, X. J. Wu, X. Feng, L. Mei, J. M. McDonald & W. C. Xiong: Regulation of the formation of osteoclastic actin rings by Pyk2 interacting with gelsolin. *J. Cell Biol.* 160, 565-575 (2003)
55. Duong, L. T., I. Nakamura, P. T. Lakkakorpi, L. Lipfert, A. J. Bett & G. A. Rodan: Inhibition of osteoclast function by adenovirus expressing antisense protein-tyrosine kinase 2. *J.Biol.Chem.* 276, 7484-7492 (2001)
56. Sanjay, A., A. Houghton, L. Neff, E. DiDomenico, C. Bardelay, E. Antoine, J. Levy, J. Gailit, D. Bowtell, W. C. Horne & R. Baron: 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.* 152, 181-195 (2001)
57. Soriano, P., C. Montgomery, R. Geske & A. Bradley: Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 64, 693-702 (1991)
58. Li, X. & H. S. Earp: Paxillin is tyrosine-phosphorylated by and preferentially associates with the calcium-dependent tyrosine kinase in rat liver epithelial cells. *J.Biol.Chem.* 272, 14341-14348 (1997)
59. Salgia, R., S. Avraham, E. Pisick, J. L. Li, S. Raja, E. A. Greenfield, M. Sattler, H. Avraham & J. D. Griffin: The related adhesion focal tyrosine kinase forms a complex with paxillin in hematopoietic cells. *J.Biol.Chem.* 271, 31222-31226 (1996)
60. Murphy, M. A., R. G. Schnall, D. J. Venter, L. Barnett, I. Bertoncello, C. B. Thien, W. Y. Langdon & D. D. Bowtell: Tissue hyperplasia and enhanced T-cell signalling via ZAP-70 in c-Cbl-deficient mice. *Mol.Cell.Biol*. 18, 4872-4882 (1998)
61. Berry, V., H. Rathod, L. B. Pulman & H. K. Datta: Immunofluorescent evidence for the abundance of focal adhesion kinase in the human and avian osteoclasts and its down regulation by calcitonin. *J.Endocrinol.* 141, R11-R15 (1994)
62. Chambers, T. J., N. A. Athanasou & K. Fuller: Effect of parathyroid hormone and calcitonin on the cytoplasmic spreading of isolated osteoclasts. *J.Endocrinol.* 102, 281-286 (1984)
63. Chambers, T. J. & C. J. Magnus: Calcitonin alters behavior of isolated osteoclasts. *J.Pathol.* 136, 27-39 (1982)
64. Tanaka, S., N. Takahashi, N. Udagawa, H. Murakami, I. Nakamura, T. Kurokawa & T. Suda: Possible involvement of focal adhesion kinase, $p125^{FAK}$, in osteoclastic bone resorption. *J.Cell.Biochem.* 58, 424-435 (1995)
65. Chellaiah, M. A., R. S. Biswas, D. Yuen, U. M. Alvarez & K. A. Hruska: Phosphatidylinositol 3,4,5-trisphosphate directs association of Src homology 2-containing signaling proteins with gelsolin. *J.Biol.Chem.* 276, 47434-47444 (2001)
66. Cunningham, C. C., T. P. Stossel & D. J. Kwiatkowski: Enhanced motility in NIH 3T3 fibroblasts that overexpress gelsolin. *Science* 251, 1233-1236 (1991)
67. Kwiatkowski, D. J.: Functions of gelsolin: motility, signaling, apoptosis, cancer. [Review] [57 refs]. *Curr.Op.Cell Biol.* 11, 103-108 (1999)
68. Sun, H. Q., M. Yamamoto, M. Mejillano & H. L. Yin: Gelsolin, a multifunctional actin regulatory protein. [Review] [74 refs]. *J.Biol.Chem*. 274, 33179-33182 (1999)
69. Hruska, K. A., F. Rolnick, M. Huskey, U. Alvarez & D Cheresh: Engagement of the osteoclast integrin alpha v beta 3 by osteopontin stimulates phosphatidylinositol 3-hydroxyl kinase activity. *Endocrinol.* 136, 2984-2992 (1995)
70. Richardson, A & J. T. Parsons: Signal transduction through integrins: a central role for focal adhesion kinase? *BioEssays* 17, 229-236 (1995)
71. Xing, Z., H. C. Chen, J. K. Nowlen, S. J. Taylor, D. Shalloway & J. L. Guan: Direct interaction of v-Src with

the focal adhesion kinase mediated by the Src SH2 domain. *Mol.Biol.Cell* 5, 413-421 (1994)
72. Chen, H. C. & J. L. Guan: Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. *Proc.Natl.Acad.Sci.U.S.A*. 91, 10148-10152 (1994)
73. Hildebrand, J. D., M. D. Schaller & J. T. Parsons: Paxillin, a tyrosine phosphorylated focal adhesion-associated protein binds to the carboxyl terminal domain of focal adhesion kinase. *Mol.Biol.Cell* 6, 637-647 (1995)
74. Polte, T. R. & S. K. Hanks: Interaction between focal adhesion kinase and Crk-associated tyrosine kinase substrate p130Cas. *Proc.Natl.Acad.Sci.U.S.A.* 92, 10678-10682 (1995)
75. Missbach, M., M. Jeschke, J. Feyen, K. Muller, M. Glatt, J. Green & M. Susa: A novel inhibitor of the tyrosine kinase Src suppresses phosphorylation of its major cellular substrates and reduces bone resorption *in vitro* and in rodent models *in vivo*. Bone 24, 437-449 (1999)
76. Chellaiah, M., C. Fitzgerald, U. Alvarez & K. Hruska: c-Src is required for stimulation of gelsolin-associated phosphatidylinositol 3-kinase. *J.Biol.Chem.* 273, 11908-11916 (1998)
77. Nakamura, I., L. Lipfert, G. A. Rodan & L. T. Duong: Convergence of alpha(v)beta(3) integrin- and macrophage colony stimulating factor-mediated signals on phospholipase Cgamma in prefusion osteoclasts. *J.Cell Biol.* 152, 361-373 (2001)
78. Nakagawa, M., T. Kaneda, T. Arakawa, S. Morita, T. Sato, T. Yomada, K. Hanada, M. Kumegawa & Y. Hakeda: Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts. *FEBS Letters* 473, 161-164 (2000)
79. Matsumoto, Y., K. Tanaka, G. Hirata, M. Hanada, S. Matsuda, T. Shuto & Y. Iwamoto: Possible involvement of the vascular endothelial growth factor-Flt-1-focal adhesion kinase pathway in chemotaxis and the cell proliferation of osteoclast precursor cells in arthritic joints. *J.Immunol.* 168, 5824-5831 (2002)

Abbreviations: FAK, focal adhesion kinase; PYK2, proline-rich tyrosine kinase 2; SH, Src homology; PI3K, phosphoinositide 3-kinase; PtdIns 4,5-P2, phosphatidylinositol 4,5-bisphosphate; PtdIns P3, phosphatidylinositol 3,4,5-triphosphate; RGD, Arg-Gly-Asp cell adhesion sequences; M-CSF, monocyte/macrophage-colony stimulating factor; RANKL, receptor activator of nuclear factor kappa B ligand; PLC-gamma, phospholipase C-gamma; VEGF, vascular endothelial growth factor

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