Citation: BoneKEy Reports **4**, Article number: 711 (2015) | doi:10.1038/bonekey.2015.80

www.nature.com/bonekey

REVIEW

Focus on the p38 MAPK signaling pathway in bone development and maintenance

Cyril Thouverey and Joseph Caverzasio

Service of Bone Diseases, Department of Internal Medicine Specialties, University Hospital of Geneva, Geneva, Switzerland.

The p38 mitogen-activated protein kinase (MAPK) signaling pathway can be activated in response to a wide range of extracellular signals. As a consequence, it can generate many different biological effects that depend on the stimulus and on the activated cell type. Therefore, this pathway has been found to regulate many aspects of tissue development and homeostasis. Recent work with the aid of genetically modified mice has highlighted the physiological functions of this pathway in skeletogenesis and postnatal bone maintenance. In this review, emphasis is given to the roles of the p38 MAPK pathway in chondrocyte, osteoblast and osteoclast biology. In particular, we describe the molecular mechanisms of p38 MAPK activation and downstream targets. The requirement of this pathway in physiological bone development and homeostasis is demonstrated by the ability of p38 MAPK to regulate master transcription factors controlling geneses and functions of chondrocytes, osteoblasts and osteoclasts.

BoneKEy Reports **4**, Article number: 711 (2015) | doi:10.1038/bonekey.2015.80

Introduction

Skeletal development starts from condensation of mesenchymal progenitor cells at the sites of future skeletal elements, and early patterning events determine where and when mesenchymal cells condense and differentiate.[1,2] A number of craniofacial bones and the lateral part of clavicles are formed through intramembranous ossification during which mesenchymal cells directly differentiate into bone-forming osteoblasts. In contrast, the axial and appendicular skeletons develop by endochondral ossification during which mesenchymal progenitors differentiate into chondrocytes to produce a cartilaginous template.[1,2] In this so-called anlagen, chondrocytes undergo a program of proliferation and progressive maturation, terminating with hypertrophic differentiation. Hypertrophic chondrocytes produce a calcified matrix and then undergo apoptosis, attracting blood vessels, osteoclasts and osteoblasts from the perichondrium to remodel the cartilaginous template into bone.[1,2] After completing its formation, bone is maintained throughout life by continuous remodeling, which is critical for its structural, mechanical and metabolic functions.[3] This active process is controlled by osteoclasts that are derived from hematopoietic progenitors and resorb bone, by osteoblasts that replace resorbed structures by an equivalent amount of new bone, and by osteocytes that are matrix-embedded terminally differentiated osteoblasts functioning as mechanical and metabolic sensors.[3]

A growing body of evidence shows that the p38 mitogen-activated protein kinase (MAPK) signaling pathway regulates many aspects of bone development and maintenance. Here, we review the functions of this signaling pathway in chondrocytes, osteoblasts and osteoclasts.

Overview of the p38 MAPK Signaling Pathway

MAPKs are evolutionarily conserved enzymes that convert extracellular stimuli into a wide range of cellular responses. Extracellular signal-regulated kinases (ERK), p38 MAPK, Jun N-terminal kinases (JNK) and ERK5 represent the four groups of the MAPK subfamily.[4,5] Among them, the p38 MAPK group is composed of four members encoded by their respective gene: p38α (encoded by *MAPK14*), p38β (encoded by *MAPK11*), p38γ (encoded by *MAPK12*) and p38δ (encoded by *MAPK13*).[4,5] p38α and p38β are closely related proteins that are expressed in most cell types and have distinct and overlapping functions. In contrast, p38γ and p38δ show very selective tissue distribution and are likely to have specialized functions.[4,5] The p38 MAPK have key roles in tissue development and homeostasis, as well as in neoplastic transformation and inflammation, as they control cell proliferation, differentiation, apoptosis, senescence and cytokine production.[4,5]

All p38 members share a similar activation loop sequence, i.e., threonine–glycine–tyrosine, which is targeted for dual phosphorylation by upstream MAPK kinases (MKK). MKK3 only

Correspondence: Professor J Caverzasio, Service of Bone Diseases, Department of Internal Medicine Specialties, University Hospital of Geneva, 64 Avenue de la Roseraie, Geneva 1205, Switzerland.
E-mail: Joseph.Caverzasio@unige.ch

Received 17 November 2014; accepted 24 April 2015; published online 10 June 2015

activates p38α, p38γ and p38δ, whereas MKK6 activates all p38 members.[4,5] In addition, MKK4, an upstream activator of JNK, can also activate p38α or p38δ in specific cell types. Finally, p38 MAPK can also be activated by an MKK-independent mechanism achieved by autophosphorylation of p38 after interaction with transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1)-binding protein 1.[4,5] Several MAPK kinase kinases (MAP3K) can activate MKK3 and MKK6, including apoptosis signal-regulating kinase 1, mixed-lineage kinase 3 (MLK3), transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1), dual-leucine-zipper-bearing kinase 1 and MAP3K4.[4,5] This diversity of MAP3K that can activate MKK3 and MKK6 provides the ability to p38 MAPK to transduce signals from a large variety of extracellular stimuli. p38 MAPK signaling can be inactivated by both generic phosphatases and dual-specificity phosphatases.[4,5] Although p38 MAPK activity is mainly controlled by phosphorylation–dephosphorylation, it can also be regulated by changes in expression levels of p38 pathway components, scaffolding proteins and autophagy.[5] The substrates of p38 MAPK are either transcription factors such as activating transcription factor 2 (ATF2), ELK1, myocyte-specific enhancer 2 (MEF2), CCAAT/enhancer-binding protein β (C/EBPβ) and C/EBP homologous protein, or other protein kinases including MAPK-activated protein kinases 2 and 3 (MAPKAPK2/3), mitogen- and stress-activated protein kinase 1 and MAPK-interacting kinases 1 and 2.[4,5]

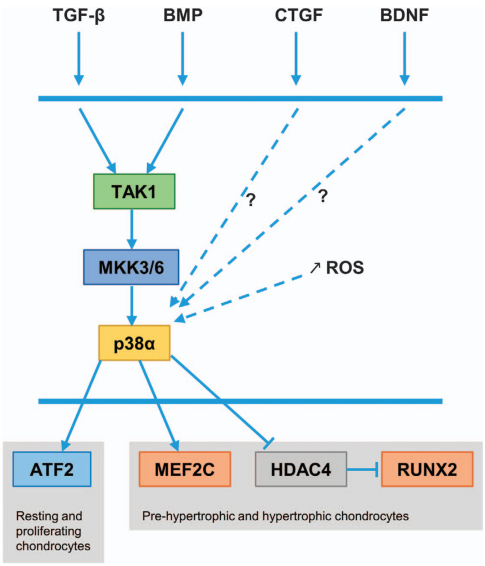

Figure 1 p38 MAPK signaling in chondrocytes. p38α is the predominant p38 member in chondrocytes. TGF-β and BMP activate p38α signaling through TAK1. CTGF and BNDF activate p38α signaling through unknown mechanisms. During terminal differentiation of chondrocytes, the level of reactive oxygen species (ROS) increases and induces p38α activation. p38α regulates ATF2 activity in resting/proliferating chondrocytes. Moreover, it regulates MEF2C, HDAC4 and RUNX2 in pre-hypertrophic and hypertrophic chondrocytes.

The p38 MAPK Signaling Pathway in Chondrocytes

Role of p38 MAPK in chondrocyte differentiation. A role of p38 MAPK in chondrogenesis has been first documented with the use of different cell culture systems and selective p38 MAPK inhibitors. The p38 MAPK phosphorylation and activity are increased during the course of the chondrogenic program, and inhibition of p38 blocks chondrocyte differentiation and cartilage nodule formation,[6] suggesting that this signaling pathway has an important role in chondrogenesis. Moreover, p38 MAPK has been demonstrated to be activated in response to and to mediate the effects of several growth factors essential for chondrocyte differentiation and function, including TGF-β, growth/differentiation factor 5 (GDF5), bone morphogenetic proteins (BMP) and connective tissue growth factor[7–10] (**Figure 1**). Indeed, the p38 MAPK pathway is involved in the regulation of *collagen 2* and *aggrecan* expressions, proteoglycan synthesis and cartilage nodule formation in response to those growth factors *in vitro*.[7–9] Notably, p38 signaling has been shown to positively regulate hypertrophic chondrocyte differentiation in a micromass system of mesenchymal cells recapitulating the entire chondrogenic program.[11] In this context, treatment of micromass cultures with p38 inhibitors results in a delay of hypertrophic differentiation as evidenced by reduced expressions of hypertrophic marker genes (*collagen 10*, *tissue nonspecific alkaline phosphatase*, *bone sialoprotein* and *matrix metalloproteinase 13*) and impaired mineralization.[11] Consistent with this finding, parathyroid hormone (PTH) and its related peptide, known to inhibit chondrocyte differentiation toward hypertrophy, have been shown to exert this negative effect by decreasing p38 MAPK activity through protein kinase C (PKC).[12] In addition, chondrocytes undergoing terminal differentiation inexorably progress toward apoptosis, and elevation of reactive oxygen species (ROS) level is probably a major mechanism governing this process.[13] It is now well recognized that those by-products of cellular oxidative metabolism can activate certain signaling pathways. Indeed, increased ROS level during chondrocyte differentiation has been found to activate ERK and p38 MAPK, resulting in chondrocyte hypertrophy[13] (**Figure 1**).

Furthermore, the brain-derived neurotrophic factor and its receptor TRKB, which regulate neuronal differentiation and survival and maintain synapse plasticity in the hippocampus and hypothalamus, have been shown to be expressed in non-neuronal cells, including growth plate chondrocytes.[14] Here, their interaction promotes hypertrophic differentiation through increased p38 MAPK activity[14] (**Figure 1**). To confirm the *in vivo* significance of these observations, the author has generated mice with targeted disruptions of *TrkB* or *p38alpha* in chondrocytes by using the *col2-cre* mouse strain.[15] Both mutant mice exhibit a similar degree of dwarfism with reduced growth-plate width and delayed hypertrophic differentiation as evidenced by altered expressions of *Sox9* and *Runx2*, two crucial transcription factors required for skeletogenesis.[15] p38α is the predominant p38 member in chondrocytes. It is localized throughout the growth-plate cartilage, but its expression is the greatest in the pre-hypertrophic zone, which is coherent with its proposed role in hypertrophic chondrocyte differentiation.[15] Interestingly, p38α phosphorylation has been found to be decreased in mice lacking *TrkB* in chondrocytes, further indicating that this pathway acts downstream of this receptor.[15] Although both mutant mice show diminished hypertrophic zone width, *p38alpha* mutant mice also exhibit narrowing and disorganization of the proliferating chondrocyte columns in growth plates, suggesting that this pathway acts downstream of other ligand/receptor interactions and also regulates other aspects of chondrocyte biology than the transition toward hypertrophy.[15]

Upstream Activators of p38 MAPK in Chondrocytes. TGF-β superfamily members such as TGF-β, BMP and GDF5 are critical regulators of chondrogenesis and exert their actions through canonical SMAD signaling and non-canonical MAPK signaling. TAK1 is a MAP3K that directs TGF-β- and BMP-induced signaling to MAPK activation[16,17] (**Figure 1**). Its *in vivo* function in cartilage development has been recently discovered by two independent groups with the use of chondrocyte-specific *Tak1* knockout mice.[16,17] TAK1 expression mainly occurs in pre-hypertrophic and hypertrophic chondrocytes during endochondral bone development.[16,17] *Tak1* mutant mice display severe chondrodysplasia with dwarfism (shorter axial and appendicular skeleton), impaired formation of secondary ossification centers and joint abnormalities. Deletion of *Tak1* in chondrocytes results in decreased chondrocyte proliferation, disorganized proliferating zone in growth plates and increased hypertrophic chondrocyte apoptosis.[16,17] Alteration of chondrocyte proliferation results from reduced expression of *Ihh* in pre-hypertrophic chondrocytes.[16,17] Only one group has observed a delayed chondrocyte hypertrophic differentiation in the absence of TAK1.[17] BMP signaling has been found to be impaired in *Tak1*-deficient chondrocytes, as shown by reduced phosphorylation of SMAD1/5/8 and p38 MAPK.[16] Therefore, it is likely that decreased p38 activity accounts for some of the skeletal abnormalities caused by *Tak1* ablation in chondrocytes.

Targets of p38 MAPK in chondrocytes. Several transcription factors that are known to be regulated by p38 MAPK are expressed in chondrocytes. For instance, p38 has been found to regulate the transcriptional activity of SOX9, the master transcription factor controlling chondrocyte differentiation.[18] Moreover, ATF2 is expressed in resting and proliferating chondrocytes during endochondral bone development. *Atf2*-deficient mice display a uniform dwarfism due to reduced chondrocyte proliferation, disorganized chondrocyte columns and impaired endochondral ossification.[19] Consistent with these observations, ATF2 regulates chondrocyte proliferation by modulating cyclin D1 levels and by inhibiting hypertrophic differentiation.[20] As ATF2 is a direct target of p38 in response to TGF-β in chondrocytes,[21] it is conceivable that p38 regulates proliferating chondrocytes through this transcription factor (**Figure 1**). On the other hand, p38 MAPK has also been found to favor hypertrophic chondrocyte differentiation both *in vitro* and *in vivo*,[11,12,15] and this, in part, by stimulating the transcriptional activity of MEF2C[11,22] (**Figure 1**). Further, it has been shown that p38 MAPK promotes histone deacetylase 4 (HDAC4) degradation and subsequent release of RUNX2 from the repressive influence of HDAC4 resulting in chondrocyte hypertrophy[23] (**Figure 1**). Therefore, it is possible that p38 signaling can exert distinct functions, depending on the developmental stage of chondrocytes and particularly on targeted transcription factors. This notion is further supported with the patterns of expression of ATF2, MEF2C and RUNX2, with ATF2 restricted to resting/proliferating chondrocytes[19] and MEF2C and RUNX2 to pre-hypertrophic/hypertrophic chondrocytes.[22,23]

The p38 MAPK Signaling Pathway in Osteoblasts

Role of p38 MAPK in osteoblast differentiation and function. Previous *in vitro* investigations using cell lines or primary osteoblasts with selective p38 inhibitors have shown that p38 MAPK regulates osteoblast differentiation, extracellular matrix deposition and mineralization in response to different osteogenic ligands such as TGF-β,[24] BMP2,[24–26] Wnt proteins,[27,28] PTH[29,30] and epinephrine[31] (**Figure 2**). p38 signaling regulates the commitment of mesenchymal cell lines to the osteoblast lineage in response to the canonical Wnt3a[27] or the non-canonical Wnt4,[28] indicating that this pathway is integrated in Wnt signaling in osteoblasts. In addition, p38 MAPK has been found to cooperate with the canonical Wnt/β–catenin pathway in response to Wnt3a, by enhancing β-catenin transcriptional activity.[27] p38 is activated by BMP in osteoblasts, and, in this situation, cooperates with the canonical SMAD signaling by favoring SMAD1 phosphorylation and its translocation to the nucleus.[26] Moreover, PTH can activate p38 MAPK through a protein kinase A (PKA)-dependent mechanism in osteoblasts.[29] In this context, p38 has been shown to be required for PTH-induced osteoblast differentiation. β-arrestin signaling, another component of PTH signaling with PKA and PKC, has been found to modulate PTH-induced p38 target gene expression.[30]

The *in vivo* function of p38 MAPK kinase signaling has been recently highlighted with different genetically modified murine models (also reviewed in Caverzasio and Thouverey[32]; Greenblatt *et al*.[33]). Three-week-old *Mkk3*$^{-/-}$ and *Mkk3*$^{-/-}$*; Mkk6*$^{+/-}$ mice display decreased mineralization of parietal and frontal bones, reduced trabecular bone volume and cortical thickness in long bones and impaired mineralization of secondary centers of ossification.[34] In comparison, *Mkk6*$^{-/-}$ mice show a low bone mass in limbs but no alteration of calvarial mineralization, indicating that those two MKKs exert anatomically selective roles.[34] Consistent with these findings and the selectivity of MKK3 and MKK6 for the four p38 members (with MKK6 activating all four members and MKK3 activating only p38α, γ and δ; **Figure 2**), 3-week-old *p38beta*-deficient mice only exhibit a low bone mass phenotype in long bones, whereas injections of cre-expressing lentivirus over the calvarium of *p38alpha*-floxed mice retard calvarial mineralization.[34] Those data further support the notion that p38α and p38β functions depend on the anatomical location and the ossification mechanism (endochondral or intramembranous) during bone development. In addition, these results also reveal that loss of p38β is not compensated by p38α and thus that p38α and p38β exert non-redundant functions. This has been confirmed by *in vitro* analyses demonstrating that p38α regulates early osteoblast differentiation, whereas p38β controls late osteoblast maturation.[34]

The p38α MAPK represents the most abundant p38 member in bone, and its specific individual contribution in bone development and postnatal maintenance has been elucidated by using the Cre-LoxP technology.[35,36] Mice in which *p38alpha* is deleted under the control of the *osterix* (*Osx*) promoter exhibit impaired mineralization of frontal and parietal bones of the calvaria, delayed posterior fontanel ossification, and abnormal development of maxilla and mandible at birth, and decreased calvarial thickness, reduced trabecular bone volume and cortical thickness in long bones at 12 weeks of age,[35] which is in accordance with abnormal skeletal development of *Mkk3*$^{-/-}$ and *Mkk3*$^{-/-}$*;Mkk6*$^{+/-}$ mice.[34] Lack of p38α in pre-osteoblasts results in defective osteoblast differentiation, as evidenced by reduced expressions of *Osx* (encoding another master transcription factor regulating osteoblast

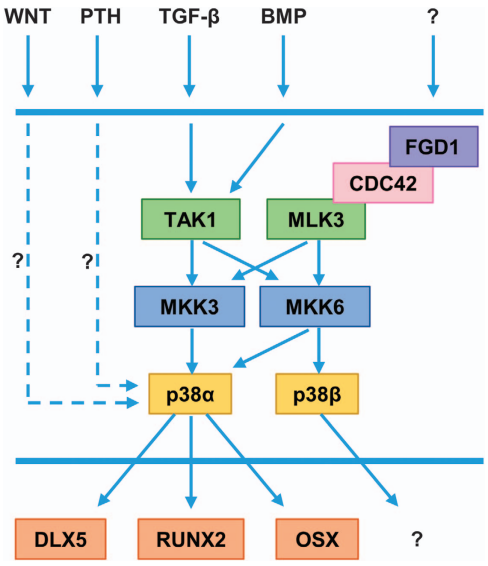

Figure 2 p38 MAPK signaling in osteoblasts. p38α regulates osteoblast differentiation and functions during bone development and adult bone homeostasis, whereas p38β regulates late osteoblast differentiation during bone development. TGF-β and BMP activate p38 signaling through TAK1-MKK3/6. PTH and WNT proteins activate p38 signaling through unknown mechanisms. MLK3 acting downstream of FGD1/CDC42 activates p38 signaling, but signals activating FGD1/CDC42 are currently unknown. MKK6 can activate both p38α and p38β, whereas MKK3 can activate only p38α. p38α positively regulates the transcriptional activity of DLX5, RUNX2 and OSX. Transcription factors regulated by p38β remain to be identified.

differentiation), *collagen 1, alkaline phosphatase, bone sialoprotein* and *osteocalcin*.[35] Mice lacking p38α in mature osteoblasts (disruption under the control of the *osteocalcin* promoter) do not display bone alterations until 5 weeks of age, but then demonstrate a progressive decrease in bone mineral density and trabecular and cortical bone mass in both axial and appendicular skeletons.[36] Those abnormalities correlate with defective osteoblast function and impaired endosteal and trabecular bone formation.[36] Those data demonstrate the critical role of p38 MAPK in postnatal bone acquisition and remodeling, which has been also confirmed by the inducible ablation of *p38alpha* in pre-osteoblasts of adult mice.[35] In contrast, 4-month-old *Mkk6*$^{-/-}$ mice, in which p38β cannot be activated, have normal osteoblast number and bone formation,[37] indicating that p38β does not have a significant role in adult bone homeostasis. Interestingly, absence of p38α in mature osteoblasts completely inhibits the bone anabolic effect of intermittent PTH treatment in mice, further supporting that p38α is an important component of PTH signaling in osteoblasts.[38] Disruption of either p38α or p38β MAPK signaling does not affect expressions of *receptor activator of nuclear factor kappa-B ligand* (*Rankl*) and *osteoprotegerin*, or bone resorption in physiological conditions.[34–36] Moreover, p38γ expression is very low in osteoblasts, whereas p38δ is expressed at the same level as p38α;[35,36] however, its role in osteoblast biology remains to be elucidated.

Upstream activators of p38 MAPK in osteoblasts. *Tak1* is expressed in bone-lining osteoblasts and in matrix-embedded osteocytes.[34] In osteoblasts, TAK1 has been shown to trigger p38 activation and only modest effects on ERK and JNK downstream of either BMP or TGF-β stimulation[34] (**Figure 2**). Mice with an *Osx-cre*-mediated excision of *Tak1* in pre-osteoblasts display clavicular hypoplasia, hypomineralization of the calvarium and delayed fontanel fusion, a phenotype resembling that of *Runx2*$^{+/-}$ mice and humans with cleidocranial dysplasia.[34] Those observations clearly indicate that TAK1 signaling is required for adequate intramembranous ossification. In addition to the clavicular and calvarial phenotypes, *Tak1* mutant mice also exhibit reduced cortical and trabecular bone mass in limbs, and decreased mineralization of secondary ossification centers.[34] This global phenotype resembles that of *Mkk3*$^{-/-}$*; Mkk6*$^{+/-}$[34] and *Osx-cre;p38alpha*$^{f/f}$ mutant mice,[35] except that the clavicular hypoplasia is not seen in those mice, suggesting that another signaling pathway contributes to clavicle development. Pre-osteoblasts lacking TAK1 fail to differentiate into mature osteoblasts and have defective function.[34]

Another MAP3K, MLK3, has been found to act downstream of faciogenital dysplasia protein 1 (FGD1) and has been identified as a physiologic regulator of osteoblastogenesis and bone development by modulating ERK and p38 MAPK activity[39] (**Figure 2**). *Mlk3*$^{-/-}$ mutant mice show dental abnormalities, defective mineralization of the calvarium and osteopenia, an equivalent skeletal phenotype in comparison with that of patients with mutations in *FGD1*. This skeletal phenotype is associated with a significant decrease in bone formation and reduced expressions of osteoblast maker genes such as *collagen 1* and *osteocalcin*.[39] In the absence of MLK3, the defective osteoblast differentiation is associated with impaired activation of ERK and p38 MAPK, and reduced RUNX2 phosphorylation.[39]

Targets of p38 MAPK in osteoblasts. The similar cleidocranial dysplasia and osteopenia observed in mice lacking TAK1 in pre-osteoblasts and *Runx2*$^{+/-}$ mice have suggested that signaling pathways downstream of TAK1 may regulate RUNX2 activity.[34] Then, p38 MAPK has been identified as a positive regulator of osteoblast differentiation and function, in part by regulating the transcriptional activity of RUNX2[34] (**Figure 2**). In fact, p38 can phosphorylate RUNX2 on Ser28, Ser31, Ser244, Ser301, Ser319 and Ser472, and thus increases its transcriptional activity by promoting its association with the transcriptional co-activator CREB-binding protein.[34] DLX5 is a member of the distal-less homeobox transcription factor family. Its expression is induced by canonical SMAD signaling downstream of BMP stimulation in osteoblasts.[40] In turn, p38 MAPK phosphorylates DLX5 and therefore increases its transcriptional activity by promoting its association with the transcriptional co-activator p300[40] (**Figure 2**). DLX5 directly stimulates osteoblast differentiation by inducing *Osx* expression. *Dlx5* knockout mice display craniofacial malformations and impaired ossification of the calvarium, but normal development of limbs and axial skeleton.[41] Thus, it is likely that p38 MAPK regulates craniofacial bone development in part through DLX5. Furthermore, p38 MAPK has also been reported to phosphorylate OSX on Ser73 and Ser77 and to stimulate its transcriptional activity by promoting its association with SWI/SNF and p300[42] (**Figure 2**). OSX and RUNX2 have also been found to interact and exert a transcriptional cooperation that is regulated by p38- and ERK-mediated phosphorylations.[43]

Modulator of p38 MAPK in osteoblasts. Finally, only one molecular mechanism involved in the regulation of p38 MAPK activity in

osteoblasts has been described so far.[44] A neighbor of *Brca1* gene protein 1 (NBR1), a receptor for selective autophagosomal degradation of ubiquitinated target proteins, has been described as a negative regulator of p38 MAPK in osteoblasts.[44] Indeed, global genetic truncation of *Nbr1* (*trNbr1*) results in progressive increase in bone mass with age due to enhanced bone formation, osteoblast differentiation and p38 activity.[44] Unlike the full-length protein, trNBR1 could not bind activated p38 MAPK and target it for degradation.[44] Interestingly, NBR1 truncation leads to negligible effect on osteoclastogenesis and bone resorption, indicating a specific regulation of p38 activity in osteoblasts.[44] Therefore, pharmacological manipulation of NBR1 may represent a conceivable option to increase bone mass in osteoporotic patients.

The p38 MAPK Signaling Pathway in Osteoclasts

Role of p38 MAPK in osteoclastogenesis. Osteoclast precursors and mature osteoclasts highly express p38α but no other p38 members.[45] Once again, first evidence highlighting the role of p38α in osteoclastogenesis has come from *in vitro* experiments employing the RAW264 cell line and primary bone marrow cells with selective p38 inhibitors and dominant negative forms of p38 signaling components.[46–48] In those cells, p38α functions downstream of RANK after stimulation by RANKL (the pro-resorptive cytokine that is necessary and sufficient for osteoclastogenesis) to stimulate osteoclast formation, maturation and bone resorption[46,47] (**Figure 3**). Inhibition of p38 MAPK completely blocks osteoclastogenesis induced by RANKL.[46,47] In osteoclast precursors, p38α functions downstream of tumor necrosis factor (TNF) receptor after stimulation by TNF-α to stimulate early osteoclast differentiation[48] (**Figure 3**). Furthermore, p38α has been shown to be a major regulator of *nuclear factor for activated T cells 1* expression (*Nfatc1*, encoding a master transcription factor regulating osteoclastogenesis).[47] Consistent with those data, mice with post-developmental deletion of the p38α-encoding gene under the control of the inducible *Mx1* promoter display reduced osteoclast number, low bone resorption and increased bone mass under physiological conditions, and are protected against TNF-α-induced arthritis and systemic bone loss.[45] Although the *Mx1-cre* deleter strain is known to induce complete gene excisions in hepatocytes and hematopoietic cells, and variable deletions in other cell types,[49] this decreased osteoclastogenesis has been shown to be cell autonomous, as bone marrow hematopoietic cells lacking p38α cannot differentiate into mature osteoclasts in response to macrophage colony-stimulating factor (M-CSF) and RANKL treatment *in vitro*.[45]

MKK3 and MKK6 have been found to be required for osteoclastogenesis *in vitro*.[37,46,50] In accordance with this, both *Mkk3*$^{-/-}$ and *Mkk6*$^{-/-}$ mice exhibit a higher trabecular bone mass with increased trabecular number and decreased trabecular spacing in comparison with control littermates at 4 months of age.[37] In those mice, osteoclast number is reduced, whereas bone formation is normal.[37] In addition, *Mkk3*$^{-/-}$ and *Mkk6*$^{-/-}$ mice are partially protected against bone loss induced by estrogen deficiency due to diminished bone resorption.[37] Absence of MKK3[37] or MKK6[46,50] in primary bone marrow cell cultures stimulated by M-CSF and RANKL results in reduced p38 MAPK activation, decreased expressions of *Nfatc1* and other osteoclast marker genes (*cathepsin K* (*CtsK*), *Oscar* and *matrix metalloproteinase 9*) and impaired bone resorption.

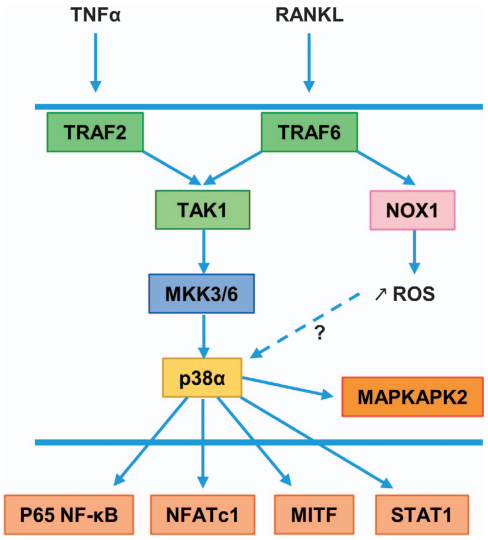

Figure 3 p38 MAPK signaling in osteoclasts. Osteoclasts and their precursors express only the p38α member. TNF-α and RANKL activate p38α MAPK signaling through TRAF2 and TRAF6, respectively. Both TRAF2 and TRAF6 regulate p38α signaling through TAK1. In response to RANKL, TRAF6 also triggers production of ROS through NAPDH oxidase 1 (NOX1), resulting in enhanced p38α activation. Finally, p38α phosphorylates the p65 NF-κB subunit, NFATc1, MITF and STAT1 to stimulate their transcriptional activity.

Upstream activators of p38 MAPK in osteoclasts. The intracellular mechanisms by which RANKL activates RANK signaling is complex (reviewed in Wada *et al.*[51]). RANK lacks intrinsic enzymatic activity in its intracellular domain and transduces signals by recruiting adapter molecules such as TNF receptor-associated factor (TRAF) family of proteins. TRAF6 has been identified as the main adapter molecule that links RANK to osteoclast differentiation and function.[51] In accordance with this notion, mice lacking TRAF6 exhibit severe osteopetrosis due to impaired osteoclast function.[52] RANKL binding to RANK induces trimerization of TRAF6, which leads to the activation of nuclear factor κB (NF-κB) and MAPK signaling.[51] Functional domains of TRAF6 involved in MAPK activation have been characterized.[53] Among the various domains investigated, the RING finger and the first zinc finger domains are required for activation of TAK1 and downstream p38 and JNK MAPK[53] (**Figure 3**). Moreover, TRAF6 has also been demonstrated to activate p38 and JNK MAPK during osteoclast differentiation by favoring ROS production through increased NAPDH oxidase activity[54] (**Figure 3**).

To date, TAK1 has been identified as the MAP3K transmitting signals between RANK/TRAF6 and NF-κB and MAPK[50,55,56] (**Figure 3**). TAK1 is activated in osteoclasts in response to RANKL and TNF-α.[50] Absence of TAK1 in primary bone marrow osteoclast precursors results in reduced activation of inhibitor of κB kinase, p38 and JNK, defective NOTCH signaling and impaired osteoclast differentiation in response to M-CSF and RANKL treatment *in vitro*.[55,56] To investigate TAK1 function in osteoclastogenesis *in vivo*, different murine models have been generated with *Tak1* inactivation in monocytes/osteoclast progenitors (under the control of the *Lysozyme M* or the

CD11b promoter) or in differentiating osteoclasts (under the control of the *CtsK* promoter).[55,56] All those mice exhibit growth retardation, severe osteopetrosis characterized by dense and brittle bone mass, and reduced osteoclast number and bone resorption,[55,56] clearly demonstrating that TAK1 is critical for physiological osteoclastogenesis and bone homeostasis. Interestingly, the osteopetrotic phenotype of those mice can be fully rescued by restoring NF-κB activation, indicating that signaling pathways generated from TAK1 (including p38) converge to enhance NF-κB activity.[56]

Targets of p38 MAPK in osteoclasts. Several downstream p38 targets involved in osteoclast differentiation and function have been characterized. RANKL induces osteoclastogenesis in great part by increasing NF-κB transcriptional activity and subsequent *Nfatc1* expression.[50,51] The p38 MAPK pathway has been found to contribute to RANKL-induced osteoclast formation by phosphorylating the p65 NF-κB subunit on Ser536 and thus increasing NF-κB transcriptional activity[50] (**Figure 3**). NFATc1, PU.1 and microphthalmia transcription factor (MITF) are important transcription factors implicated in osteoclast differentiation and can interact with each other to synergistically enhance osteoclast marker gene expressions.[47,57] Activated p38 MAPK in response to RANKL has been shown to phosphorylate NFATc1 (**Figure 3**), which leads to translocation of NFATc1 in osteoclast nuclei, its association with PU.1 and increased *CtsK* and *tartrate-resistant acid phosphatase type 5* (*Acp5*) expressions.[47,57] In addition, p38 MAPK can directly phosphorylate MITF on Ser307 following RANKL stimulation (**Figure 3**), thus leading to increased MITF transactivation and enhanced *CtsK* and *Acp5* expressions.[58] Monokine induced by interferon γ (MIG) is secreted by osteoclast precursors upon RANKL stimulation and stimulates osteoclast adhesion and migration.[59] It has been demonstrated that MIG induction by RANKL is dependent on signal transducer and activator of transcription 1 (STAT1) activity and that STAT1 phosphorylation on Ser727 by p38 is critical in this context[59] (**Figure 3**). Finally, p38 MAPK is known to activate downstream kinases such as MAPKAPK2[4,5] (**Figure 3**). Interestingly, bone marrow osteoclast precursors lacking MAPKAPK2 fail to differentiate into mature osteoclasts in response to M-CSF and RANKL treatment *in vitro*, as evidenced by reduced expression of *Acp5*, *CtsK*, *Oscar* and *Mmp9*, and impaired DNA-binding capacity of c-FOS and NFATc1 in promoters of osteoclast-specific genes.[60] Coherent with that, mice lacking MAPKAPK2 show increased trabecular bone mass and cortical thickness, fewer osteoclasts and lower bone resorption compared with control littermates.[60] Altogether, those data highlight the critical role of p38α in osteoclastogenesis and bone remodeling.

Conclusion

A large body of evidence shows that the p38 MAPK signaling pathway is critical for skeleton development and bone homeostasis (**Table 1**). Mutations in genes affecting the p38 pathway can cause developmental bone disorders such as chondrodysplasia, cleidocranial dysplasia or faciogenital dysplasia. In addition, this signaling pathway is altered in the context of osteoporosis, inflammatory osteolysis and osteopetrosis. p38α is the most abundant p38 member in bone cells, but direct therapeutic intervention on this MAPK is unthinkable because of its ubiquitous nature and pleiotropic functions.

Table 1 Skeletal phenotypes of mice with genetic deletion of genes encoding components of the p38 MAPK signaling pathway

Affected cells	*Deleted kinase*	*Type of deletion*	*Skeletal phenotype*	*Cell defects*	*Ref.*
Chondrocytes	p38α	*Col2-cre*	Dwarfism, narrowing/disorganization of proliferative and hypertrophic zones in growth plates	Delayed hypertrophic differentiation	15
	TAK1	*Col2-cre*	Severe chondrodysplasia with dwarfism, impaired formation of secondary ossification centers, joint abnormalities	Decreased proliferation, increased apoptosis Delayed hypertrophic differentiation	16,17 17
Osteoblasts	p38α	*Osx-cre*, inducible	Defective mineralization of craniofacial bones at birth, low bone mass in adulthood Decreased bone formation when deleted during adulthood	Impaired early osteoblast differentiation	35
		Ocn-cre	Decreased bone formation, low bone mass getting worse with age	Defective osteoblast function	36
	p38β	Global KO	Low bone mass only in limbs at 3 weeks of age	Impaired late osteoblast maturation	34
	MKK3	Global KO	Decreased calvarial mineralization, low bone mass at 3 weeks of age	Impaired early osteoblast differentiation	34
	MKK6	Global KO	Low bone mass only in limbs at 3 weeks of age	Impaired late osteoblast maturation	34
	TAK1	*Osx-cre*, inducible	Cleidocranial dysplasia, decreased mineralization of secondary ossification centers, low bone mass at 3 weeks of age	Impaired early osteoblast differentiation	34
	MLK3	Global KO	Dental abnormalities, defective calvarial mineralization, osteopenia	Impaired early osteoblast differentiation	39
Osteoclasts	p38α	*Mx-cre*, inducible	Increased bone mass	Impaired osteoclastogenesis	45
	MKK3	Global KO	High trabecular bone mass at 16 weeks of age	Impaired osteoclastogenesis	37
	TAK1	*LysM-cre* *CtsK-cre*	Growth retardation, severe osteopetrosis	Impaired osteoclastogenesis	55,56

Abbreviations: *CtsK*, *cathepsin K*; KO, knockout; MKK, mitogen-activated protein kinase kinase; MLK, mixed-lineage kinase; *Osx*, *osterix*; TAK1, transforming growth factor-β-activated kinase 1.

Therefore, an accurate knowledge of p38 MAPK function and regulation is needed to potentially identify therapeutic targets with the goal of modulating this pathway in bone. In particular, its molecular regulatory mechanisms (inhibiting phosphatases, scaffold proteins, molecular targeting to autophaghy), which generally function in a cell type-specific manner, may represent better targets than p38α MAPK itself. For instance, NBR1, which negatively regulates p38 activity specifically in osteoblasts, may represent such existing therapeutic targets to increase bone mass in osteoporotic patients.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Olsen BR, Reginato AM, Wang W. Bone development. *Annu Rev Cell Dev Biol* 2000; **16**: 191–220.
2. Karsenty G, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. *Dev Cell* 2002; **2**: 389–406.
3. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. *J Biol Chem* 2010; **285**: 25103–25108.
4. Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. *Cell Res* 2005; **15**: 11–18.
5. Cuadrado A, Nebreda AR. Mechanisms and functions of p38 MAPK signaling. *Biochem J* 2010; **429**: 403–417.
6. Oh CD, Chang SH, Yoon YM, Lee SJ, Lee YS, Kang SS *et al.* Opposing role of mitogen-activated protein kinase subtypes, erk-1/2 and p38, in the regulation of chondrogenesis of mesenchymes. *J Biol Chem* 2000; **275**: 5613–5619.
7. Nakamura K, Shirai T, Morishita S, Uchida S, Saeki-Miura K, Makishima F. p38 mitogen-activated protein kinase functionally contributes to chondrogenesis induced by growth/differentiation factor-5 in ATDC5 cells. *Exp Cell Res* 1999; **250**: 351–363.
8. Yosimichi G, Nakanishi T, Nishida T, Hattori T, Takano-Yamamoto T, Takigawa M. CTGF/Hcs24 induces chondrocyte differentiation through a p38 mitogen-activated protein kinase (p38MAPK), and proliferation through a p44/42 MAPK/extracellular-signal regulated kinase (ERK). *Eur J Biochem* 2001; **268**: 6058–6065.
9. Tuli R, Tuli S, Nandi S, Huang X, Manner PA, Hozack WJ *et al.* Transforming growth factor-beta-mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and mitogen-activated protein kinase and Wnt signaling cross-talk. *J Biol Chem* 2003; **278**: 41227–41236.
10. Zuzarte-Luís V, Montero JA, Rodriguez-León J, Merino R, Rodríguez-Rey JC, Hurlé JM. A new role for BMP5 during limb development acting through the synergic activation of Smad and MAPK pathways. *Dev Biol* 2004; **272**: 39–52.
11. Stanton LA, Sabari S, Sampaio AV, Underhill TM, Beier F. p38 MAP kinase signalling is required for hypertrophic chondrocyte differentiation. *Biochem J* 2004; **378**: 53–62.
12. Zhen X, Wei L, Wu Q, Zhang Y, Chen Q. Mitogen-activated protein kinase p38 mediates regulation of chondrocyte differentiation by parathyroid hormone. *J Biol Chem* 2001; **276**: 4879–4885.
13. Morita K, Miyamoto T, Fujita N, Kubota Y, Ito K, Takubo K *et al.* Reactive oxygen species induce chondrocyte hypertrophy in endochondral ossification. *J Exp Med* 2007; **204**: 1613–1623.
14. Hutchison MR. BDNF alters ERK/p38 MAPK activity ratios to promote differentiation in growth plate chondrocytes. *Mol Endocrinol* 2012; **26**: 1406–1416.
15. Hutchison MR. Mice with a conditional deletion of the neuro trophin receptor TrkB are dwarfed, and are similar to mice with a MAPK14 deletion. *PLoS ONE* 2013; **8**: e66206.
16. Shim JH, Greenblatt MB, Xie M, Schneider MD, Zou W, Zhai B *et al.* TAK1 is an essential regulator of BMP signalling in cartilage. *EMBO J* 2009; **28**: 2028–2041.
17. Gunnell LM, Jonason JH, Loiselle AE, Kohn A, Schwarz EM, Hilton MJ *et al.* TAK1 regulates cartilage and joint development via the MAPK and BMP signaling pathways. *J Bone Miner Res* 2010; **25**: 1784–1797.
18. Zhang R, Murakami S, Coustry F, Wang Y, de Crombrugghe B. Constitutive activation of MKK6 in chondrocytes of transgenic mice inhibits proliferation and delays endochondral bone formation. *Proc Natl Acad Sci USA* 2006; **103**: 365–370.
19. Reimold AM, Grusby MJ, Kosaras B, Fries JW, Mori R, Maniwa S *et al.* Chondrodysplasia and neurological abnormalities in ATF-2-deficient mice. *Nature* 1996; **379**: 262–265.
20. Beier F, Lee RJ, Taylor AC, Pestell RG, LuValle P. Identification of the cyclin D1 gene as a target of activating transcription factor 2 in chondrocytes. *Proc Natl Acad Sci USA* 1999; **96**: 1433–1438.
21. Ionescu AM, Schwarz EM, Zuscik MJ, Drissi H, Puzas JE, Rosier RN *et al.* ATF-2 cooperates with Smad3 to mediate TGF-beta effects on chondrocyte maturation. *Exp Cell Res* 2003; **288**: 198–207.
22. Arnold MA, Kim Y, Czubryt MP, Phan D, McAnally J, Qi X *et al.* MEF2C transcription factor controls chondrocyte hypertrophy and bone development. *Dev Cell* 2007; **12**: 377–389.
23. Zhou J, Li P, Chen Q, Wei X, Zhao T, Wang Z *et al.* Mitogen-activated protein kinase p38 induces HDAC4 degradation in hypertrophic chondrocytes. *Biochim Biophys Acta* 2015; **1853**: 370–376.
24. Lai CF, Cheng SL. Signal transductions induced by bone morphogenetic protein-2 and transforming growth factor-beta in normal human osteoblastic cells. *J Biol Chem* 2002; **277**: 15514–15522.
25. Guicheux J, Lemonnier J, Ghayor C, Suzuki A, Palmer G, Caverzasio J. Activation of p38 mitogen-activated protein kinase and c-Jun-NH2-terminal kinase by BMP-2 and their implication in the stimulation of osteoblastic cell differentiation. *J Bone Miner Res* 2003; **18**: 2060–2068.
26. Nöth U, Tuli R, Seghatoleslami R, Howard M, Shah A, Hall DJ *et al.* Activation of p38 and Smads mediates BMP-2 effects on human trabecular bone-derived osteoblasts. *Exp Cell Res* 2003; **291**: 201–211.
27. Caverzasio J, Manen D.. Essential role of Wnt3a-mediated activation of mitogen-activated protein kinase p38 for the stimulation of alkaline phosphatase activity and matrix mineralization in C3H10T1/2 mesenchymal cells. *Endocrinology* 2007; **148**: 5323–5330.
28. Chang J, Sonoyama W, Wang Z, Jin Q, Zhang C, Krebsbach PH *et al.* Noncanonical Wnt-4 signaling enhances bone regeneration of mesenchymal stem cells in craniofacial defects through activation of p38 MAPK. *J Biol Chem* 2007; **282**: 30938–30948.
29. Rey A, Manen D, Rizzoli R, Ferrari SL, Caverzasio J. Evidences for a role of p38 MAP kinase in the stimulation of alkaline phosphatase and matrix mineralization induced by parathyroid hormone in osteoblastic cells. *Bone* 2007; **41**: 59–67.
30. Bianchi EN, Ferrari SL. Beta-arrestin2 regulates parathyroid hormone effects on a p38 MAPK and NFkappaB gene expression network in osteoblasts. *Bone* 2009; **45**: 716–725.
31. Suzuki A, Palmer G, Bonjour JP, Caverzasio J. Regulation of alkaline phosphatase activity by p38 MAP kinase in response to activation of Gi protein-coupled receptors by epinephrine in osteoblast-like cells. *Endocrinology* 1999; **140**: 3177–3182.
32. Caverzasio J, Thouverey C. Discovering the multiple roles of the p38 pathway in bone biology. *IBMS BoneKEy* 2010; **7**: 458–461.
33. Greenblatt MB, Shim JH, Glimcher LH. Mitogen-activated protein kinase pathways in osteoblasts. *Annu Rev Cell Dev Biol* 2013; **29**: 63–79.
34. Greenblatt MB, Shim JH, Zou W, Sitara D, Schweitzer M, Hu D *et al.* The p38 MAPK pathway is essential for skeletogenesis and bone homeostasis in mice. *J Clin Invest* 2010; **120**: 2457–2473.
35. Rodríguez-Carballo E, Gámez B, Sedó-Cabezón L, Sánchez-Feutrie M, Zorzano A, Manzanares-Céspedes C *et al.* The p38α MAPK function in osteoprecursors is required for bone formation and bone homeostasis in adult mice. *PLoS ONE* 2014; **9**: e102032.
36. Thouverey C, Caverzasio J. The p38α MAPK positively regulates osteoblast function and postnatal bone acquisition. *Cell Mol Life Sci* 2012; **69**: 3115–3125.
37. Boyle DL, Hammaker D, Edgar M, Zaiss MM, Teufel S, David JP *et al.* Differential roles of MAPK kinases MKK3 and MKK6 in osteoclastogenesis and bone loss. *PLoS One* 2014; **9**: e84818.
38. Thouverey C, Caverzasio J. Osteoblast-specific ablation of *p38a* blunts the bone anabolic activity of parathyroid hormone. *Bone Abstracts* 2014; **3**: HT2.
39. Zou W, Greenblatt MB, Shim JH, Kant S, Zhai B, Lotinun S *et al.* MLK3 regulates bone development downstream of the faciogenital dysplasia protein FGD1 in mice. *J Clin Invest* 2011; **121**: 4383–4392.
40. Ulsamer A, Ortuño MJ, Ruiz S, Susperregui AR, Osses N, Rosa JL *et al.* BMP-2 induces Osterix expression through up-regulation of Dlx5 and its phosphorylation by p38. *J Biol Chem* 2008; **283**: 3816–3826.
41. Acampora D, Merlo GR, Paleari L, Zerega B, Postiglione MP, Mantero S *et al.* Craniofacial, vestibular and bone defects in mice lacking the Distal-less-related gene Dlx5. *Development* 1999; **126**: 3795–3809.
42. Ortuño MJ, Ruiz-Gaspà S, Rodríguez-Carballo E, Susperregui AR, Bartrons R, Rosa JL *et al.* p38 regulates expression of osteoblast-specific genes by phosphorylation of osterix. *J Biol Chem* 2010; **285**: 31985–31994.
43. Artigas N, Ureña C, Rodríguez-Carballo E, Rosa JL, Ventura F. Mitogen-activated protein kinase (MAPK)-regulated interactions between Osterix and Runx2 are critical for the transcriptional osteogenic program. *J Biol Chem* 2014; **289**: 27105–27117.
44. Whitehouse CA, Waters S, Marchbank K, Horner A, McGowan NW, Jovanovic JV *et al.* Neighbor of Brca1 gene (Nbr1) functions as a negative regulator of postnatal osteoblastic bone formation and p38 MAPK activity. *Proc Natl Acad Sci USA* 2010; **107**: 12913–12918.
45. Böhm C, Hayer S, Kilian A, Zaiss MM, Finger S, Hess A *et al.* The alpha-isoform of p38 MAPK specifically regulates arthritic bone loss. *J Immunol* 2009; **183**: 5938–5947.
46. Matsumoto M, Sudo T, Saito T, Osada H, Tsujimoto M. Involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-kappa B ligand (RANKL). *J Biol Chem* 2000; **275**: 31155–31161.
47. Matsumoto M, Kogawa M, Wada S, Takayanagi H, Tsujimoto M, Katayama S *et al.* Essential role of p38 mitogen-activated protein kinase in cathepsin K gene expression during osteoclastogenesis through association of NFATc1 and PU.1. *J Biol Chem* 2004; **279**: 45969–45979.
48. Matsumoto M, Sudo T, Maruyama M, Osada H, Tsujimoto M. Activation of p38 mitogen-activated protein kinase is crucial in osteoclastogenesis induced by tumor necrosis factor. *FEBS Lett* 2000; **486**: 23–28.
49. Kühn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. *Science* 1995; **269**: 1427–1429.

50. Huang H, Ryu J, Ha J, Chang EJ, Kim HJ, Kim HM *et al.* Osteoclast differentiation requires TAK1 and MKK6 for NFATc1 induction and NF-kappaB transactivation by RANKL. *Cell Death Differ* 2006; **13**: 1879–1891.
51. Wada T, Nakashima T, Hiroshi N, Penninger JM. RANKL-RANK signaling in osteoclastogenesis and bone disease. *Trends Mol Med* 2006; **12**: 17–25.
52. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A *et al.* TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev* 1999; **13**: 1015–1024.
53. Kobayashi N, Kadono Y, Naito A, Matsumoto K, Yamamoto T, Tanaka S *et al.* Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. *EMBO J* 2001; **20**: 1271–1280.
54. Lee NK, Choi YG, Baik JY, Han SY, Jeong DW, Bae YS *et al.* A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* 2005; **106**: 852–859.
55. Lamothe B, Lai Y, Xie M, Schneider MD, Darnay BG. TAK1 is essential for osteoclast differentiation and is an important modulator of cell death by apoptosis and necroptosis. *Mol Cell Biol* 2013; **33**: 582–595.
56. Swarnkar G, Karuppaiah K, Mbalaviele G, Chen TH, Abu-Amer Y. Osteopetrosis in TAK1-deficient mice owing to defective NF-κB and NOTCH signaling. *Proc Natl Acad Sci USA* 2015; **112**: 154–159.
57. Sharma SM, Bronisz A, Hu R, Patel K, Mansky KC, Sif S *et al.* MITF and PU.1 recruit p38 MAPK and NFATc1 to target genes during osteoclast differentiation. *J Biol Chem* 2007; **282**: 15921–15929.
58. Mansky KC, Sankar U, Han J, Ostrowski MC. Microphthalmia transcription factor is a target of the p38 MAPK pathway in response to receptor activator of NF-kappa B ligand signaling. *J Biol Chem* 2002; **277**: 11077–11083.
59. Kwak HB, Lee SW, Jin HM, Ha H, Lee SH, Takeshita S *et al.* Monokine induced by interferon-gamma is induced by receptor activator of nuclear factor kappa B ligand and is involved in osteoclast adhesion and migration. *Blood* 2005; **105**: 2963–2969.
60. Braun T, Lepper J, Ruiz Heiland G, Hofstetter W, Siegrist M, Lezuo P *et al.* Mitogen-activated protein kinase 2 regulates physiological and pathological bone turnover. *J Bone Miner Res* 2013; **28**: 936–947.