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COMMENTARIES

Discovering the Multiple Roles of the p38 Pathway in Bone Biology

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Commentary on: Greenblatt MB, Shim JH, Zou W, Sitara D, Schweitzer M, Hu D, Lotinun S, Sano Y, Baron R, Park JM, Arthur S, Xie M, Schneider MD, Zhai B, Gygi S, Davis R, Glimcher LH. The p38 MAPK pathway is essential for skeletogenesis and bone homeostasis in mice. *J Clin Invest.* 2010 Jul 1;120(7):2457-73.

During bone development and remodeling, osteoblast responses to extracellular stimuli are mediated by signaling pathways such as the mitogen-activated protein (MAP) kinase pathway. Four distinct subgroups within the MAP kinase family have been described: 1) extracellular signal-regulated kinases (ERK1/2); 2) c-Jun N-terminal protein kinases (JNK1/2); 3) ERK/big MAP kinase 1 (BMK1); and 4) the p38 group of protein kinases. Several *in vitro* studies using selective inhibitors of p38 have shown that this pathway is important for the regulation of alkaline phosphatase activity and matrix mineralization in response to several osteogenic ligands such as BMP-2 and TGF-β (1;2), epinephrine (3), PTH (4) and Wnt proteins (5). A recent report by Greenblatt *et al*. in the *Journal of Clinical Investigation* addresses for the first time the role of the p38 pathway in osteoblast biology by targeting the TGF-β-activated kinase 1 (TAK1)-MKK3/6-p38 pathway with a Cre-*lox* system (6). The authors provide compelling evidence for an *in vivo* function of the TAK1-MKK3/6 pathway in controlling skeletogenesis and bone homeostasis in mice.

The p38 MAP kinase group is composed of p38α, p38β, p38γ and p38δ. Among the four members, p38α is the most studied, and has been implicated in cellular processes such as proliferation, differentiation, apoptosis, senescence, and inflammation. The activity of p38 is regulated by cellular mechanisms that control its phosphorylation and dephosphorylation. The major phosphorylation/activation mechanism is mediated by MKK3/MKK6. MKK3 only activates p38α, p38γ and p38δ whereas MKK6 activates all members of the p38 group. MKK4, an upstream kinase of JNK, can also activate p38α or p38δ in specific cell types. Finally, p38 can also be activated by an MKK-independent mechanism achieved by autophosphorylation of p38 after interaction with TAB1 (7). There are several upstream kinases that can activate MKK3/MKK6 including TAK1, ASK1, DLK and MEKK4 (8). The contribution of each of these kinases in osteoblast biology is unknown. Of these, TAK1 is a MAP3K that was originally characterized by Matsumoto and co-workers, who demonstrated that the kinase activity of TAK1 is activated in response to TGF-β and BMP to regulate p38 MAPK and gene transcription (9). Then, additional studies have reported that TAK1 also activates JNK and I-κ B kinase complex (IKK). p38 and JNK control the transcription factor activator protein-1 (AP-1) and IKK controls nuclear factor κ B (NF-κB) (10). More recently, TAK1 has also been shown to be implicated in activation of a tumor suppressor protein, the LKB1 serine/threonine kinase (11) that in turn can activate a family of kinases related to adenosine monophosphate (AMP)-activated protein kinase (AMPK) regulating cell metabolism, growth and polarity.

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From the initial information that TAK1 is an important activator of the p38 pathway in response to TGF-β and BMPs (9), the group led by Laurie Glimcher investigated the *in vivo* function of this pathway in osteoblast biology using osteoblast-specific deletion of TAK1 in mice (6). Essentially, these authors found that deletion of TAK1 in early differentiating osteoblasts (*Osx*-cre system) resulted in clavicular hypoplasia and delayed fontanelle fusion (hypomineralization of the calvarium), a phenotype similar to the cleidocranial dysplasia observed in human haploinsufficiency for the transcription factor runt-related transcription factor 2 (Runx2). Immunohistochemistry analysis indicated that TAK1 is expressed in the osteogenic front and in osteocytes, suggesting various functions of this molecule in controlling early osteoblast differentiation and osteocyte-mediated control of bone homeostasis. In addition to the clavicular and calvarial phenotypes, *TAK1*-floxed mice expressing an *Osx*-cre transgene (*TAK1*^{osx} mice) displayed a marked reduction in trabecular and cortical bone mass in the long bones at three weeks of age. This phenotype was associated with a substantial reduction in the mineralization of the secondary center of ossification above the growth plate. Histomorphometric analysis and metabolic labeling of bone formation demonstrated an important reduction in osteoblast number (-60 to -70%), osteoid volume (-70%) and mineralizing surface area, indicating a profound impairment in osteoblastic function. There were no significant changes in osteoclast number, activity and bone resorption associated with the alterations in bone formation parameters. The lower number of osteoblasts was associated with a substantial decrease in marker genes of cell differentiation such as *Osx*, *Alp*, *Col1*, and *Ocn* with a 20% decrease in mRNA and protein levels of Runx2. The molecular mechanism involved in the marked decrease in osteoblastic function was then investigated in primary cultured osteoblasts derived from calvaria of *TAK1*-floxed mice infected with either an empty vector or a cre-expressing lentivirus. Several signaling pathways were investigated including the SMADs, TAK1, MKK3/6, p38, ERK1/2, and JNK1/2 in response to BMP and TGF-β. In TAK1-deficient osteoblasts, activation of SMAD was slightly reduced and Tak1, MKK3/6 and p38 were not activated in response to BMP2 whereas ERK1/2 and JNK1/2 activation were not affected. Upon TGF-β stimulation (data presented in supplemental material), TAK1 and MKK3/6 activation were suppressed but, surprisingly, p38 activity was elevated in Tak1-deficient osteoblasts, indicating that the p38 pathway can still be activated by a TAK1-independent mechanism in these cells.

To further assess the contribution of the p38 pathway in mediating the observed *Tak1*^{osx} phenotype, the authors used several approaches to selectively inhibit the p38 pathway *in vitro* and *in vivo*. The use of either a selective p38 inhibitor in primary calvarial osteoblasts or the expression of a kinase-inactive MKK6 mutant in mesenchymal stem cells inhibited osteoblast differentiation, confirming previous observations that the p38 pathway is a critical regulator of osteoblast differentiation *in vitro*. To determine the *in vivo* function of direct activators of p38 in bone biology, the authors used *MKK3*(-/-), *MKK6*(-/-) and *MKK3*(-/-)/MKK6*(+/-)* mice. Interestingly, the bone phenotype was different in *MKK3*(-/-) and *MKK6*(-/-) 25-day-old mice. *MKK3*(-/-) mice displayed a calvarial and a femoral phenotype whereas *MKK6*(-/-) mice presented essentially a femoral phenotype. Since it has been reported that MKK3 only activates p38α, p38γ and p38δ, this observation suggests that MKK3 and MKK6 may play anatomically selective roles with p38α but not p38β being involved in calvarial and both p38 kinases in long bone mineralization. Also, hypoplasia of the clavicle seen in *Tak1*^{osx} mice was not seen in *MKK3*(-/-)/*MKK6*(+/-) mice, suggesting that another pathway mediates this effect or that a more complete blockade of the p38 MAPK pathway is necessary to prevent the development of the clavicle, as mentioned by the authors. Confirmation that p38α is involved in calvarial mineralization was demonstrated using the injection of a cre-expressing lentivirus over the calvarium of *p38*-floxed mice. Interesting data on the bone phenotype of *p38β*(-/-) mice were also

reported. These mice showed a substantial decrease in long bone mass and a more modest effect on the calvarium. This observation is surprising since it suggests that the loss of p38β is not compensated by p38α and that these two MAPKs may have different effects in long bone development. This hypothesis needs confirmation in studies using selective ablation of each p38 MAP kinase in osteoblasts. Different cellular effects of p38α and p38β were presented in calvarial osteoblasts with p38α but not p38β influencing alkaline phosphatase activity whereas both MAPKs affected *Osx*, *Ocn* and *Col1*. This observation and further *in vitro* analyses suggested that p38α affects early osteoblast differentiation and p38β only late differentiation. Consistent with this latter proposition, MKK6 and p38β were found to be upregulated during the course of osteoblast differentiation. To prove that the p38 MAPK pathway is involved in mediating the effect of TAK1 to promote osteoblast differentiation, the authors expressed either a constitutively active or a kinase-dead mutant of MKK6 in WT and TAK1-deficient osteoblasts. Expression of the constitutively active MKK6 partially rescued the TAK1 phenotype, implicating the TAK1-p38 pathway in osteoblast differentiation with the likely involvement of other mechanisms.

Since the phenotype of *TAK1*^{osx} mice was similar to that of the *Runx2*(+/-) mice, the authors investigated the role of p38 in the activation of Runx2. Using different cellular and molecular experimental approaches, they provided compelling evidence that p38α can phosphorylate Runx2 at three serine (S17, S261 and S298) residues and that the phosphorylation of these residues is critical for the transcriptional activity of Runx2. Changes in this transcriptional activity involved alteration in Runx2 DNA binding activity and association with the coactivator CREB binding protein.

This work provides the first description of an *in vivo* bone phenotype for p38β-deficient mice. The selectivity of p38β function for bone suggests that p38β-specific inhibitors and agonists may be excellent tools to pharmacologically modulate bone formation. In addition to its effects on osteoblastogenesis, the p38 pathway has also been implicated in the regulation of osteoclasts influencing both the production and action of several cytokines involved in osteoclastogenesis (12;13). In this context, since p38α is the only member expressed in osteoclast precursors, selective p38α inhibitors have been found to successfully prevent osteoclast-mediated inflammatory osteolysis (14) and bone loss induced by estrogen deficiency (15). Therefore, the clarification of the *in vivo* function of p38 MAP kinases in osteoblastogenesis is an objective of prime importance to evaluate the possible negative effects of p38 inhibitors, potentially used to treat rheumatoid arthritis or postmenopausal osteoporosis, on bone formation and homeostasis.

In summary, the results presented in the study by Greenblatt *et al*. represent the first genetic evidence for a role of TAK1 in bone biology. The authors also provide strong evidence that the role of TAK1 is mainly mediated by the MKK3/MKK6-p38 pathway. However, the contribution of other signaling mechanisms triggered by TAK1 cannot be ruled out. Furthermore, the p38 pathway is also implicated in signal transduction by osteogenic ligands that differ from BMPs and TGF-β. Therefore, additional studies are required to precisely determine the apparently different functions of p38α and p38β in controlling bone development and homeostasis.

Conflict of Interest: None reported.

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References

1. Lee KS, Hong SH, Bae SC. Both the Smad and p38 MAPK pathways play a crucial role in Runx2 expression following induction by transforming growth factor-beta and bone morphogenetic protein. *Oncogene*. 2002 Oct 17;21(47):7156-63.

2. 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 Nov;18(11):2060-8.

3. 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 Jul;140(7):3177-82.

4. 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 Jul;41(1):59-67.

5. 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 Nov;148(11):5323-30.

6. Greenblatt MB, Shim JH, Zou W, Sitara D, Schweitzer M, Hu D, Lotinun S, Sano Y, Baron R, Park JM, Arthur S, Xie M, Schneider MD, Zhai B, Gygi S, Davis R, Glimcher LH. The p38 MAPK pathway is essential for skeletogenesis and bone homeostasis in mice. *J Clin Invest*. 2010 Jul 1;120(7):2457-73.

7. Ge B, Gram H, Di Padova F, Huang B, New L, Ulevitch RJ, Luo Y, Han J. MAPKK-independent activation of p38alpha mediated by TAB1-dependent autophosphorylation of p38alpha. *Science*. 2002 Feb 15;295(5558):1291-4.

8. Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. *Cell Res*. 2005 Jan;15(1):11-8.

9. Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, Taniguchi T, Nishida E, Matsumoto K. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science*. 1995 Dec 22;270(5244):2008-11.

10. Landström M. The TAK1-TRAF6 signalling pathway. *Int J Biochem Cell Biol*. 2010 May;42(5):585-9.

11. Adhikari A, Xu M, Chen ZJ. Ubiquitin-mediated activation of TAK1 and IKK. *Oncogene*. 2007 May 14;26(22):3214-26.

12. Li X, Udagawa N, Itoh K, Suda K, Murase Y, Nishihara T, Suda T, Takahashi N. p38 MAPK-mediated signals are required for inducing osteoclast differentiation but not for osteoclast function. *Endocrinology*. 2002 Aug;143(8):3105-13.

13. Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. IL-1 mediates TNF-induced osteoclastogenesis. *J Clin Invest*. 2005 Feb;115(2):282-90.

14. Mbalaviele G, Anderson G, Jones A, De Ciechi P, Settle S, Mnich S, Thiede M, Abu-Amer Y, Portanova J, Monahan J. Inhibition of p38 mitogen-activated protein kinase prevents inflammatory bone destruction. *J Pharmacol Exp Ther*. 2006 Jun;317(3):1044-53.

15. Caverzasio J, Higgins L, Ammann P. Prevention of trabecular bone loss induced by estrogen deficiency by a selective p38alpha inhibitor. *J Bone Miner Res*. 2008 Sep;23(9):1389-97.