BoneKEy-Osteovision. 2006 April;3(4):5-11
http://www.bonekey-ibms.org/cgi/content/full/ibmske;3/4/5
DOI: 10.1138/20060205

PERSPECTIVES

BMP and Its Antagonists

Masaki Noda

Tokyo Medical and Dental University, Tokyo, Japan

Abstract

Bone morphogenetic protein (BMP) is necessary for the development of mesenchymal tissue and mesoderm formation during development. BMP2 and BMP4 play critical roles in morphogenesis. On the other hand, BMP5, BMP6 and BMP7, which were discovered more recently, likely serve as specific modulators of the development of other tissues. There are several inhibitory molecules that seem able to target BMP functioning, including SOST, Tob, CIZ and Noggin. These molecules appear to help determine the levels of bone mass in adult mice. Further analysis is necessary to elucidate the specific functions of BMP inhibitors and BMPs in adult bone. *BoneKEy-Osteovision*. 2006 April;3(4):5-11.
©2006 International Bone and Mineral Society

Bone morphogenetic protein (BMP) was first identified by Urist in the 1960s as a protein within bone matrix that could induce bone formation *in vivo* when implanted in muscle or subcutaneous tissue (1). However, the molecular details of the protein's function were not clearly defined until the later cloning of BMP genes. In the late 1980s, an American company, Genetics Institute, cloned several human BMP genes (2). Sequence analysis based on the cDNAs for BMP1, 2, 3 and 4 showed that BMP1 is not related to other BMP family members. However, the other BMPs were found to exhibit homology among their sequences, and most importantly, the BMP family members showed the presence of conserved cysteine residues that are characteristic of members of the TGF-β superfamily. Following the discovery of the BMP genes, receptors for BMPs were identified and genes encoding these receptors were cloned (3). Subsequently, Smad molecules that mediate BMP signaling have been identified. The Smad family members have been classified into R-Smad, C-Smad, and I-(inhibitory) Smads (4-8).

BMP family members have been shown to be expressed during development. Each of the different BMPs, such as BMP2, 4, 5, 6 and 7 is expressed in a distinctive pattern (5). Thus, although all BMPs are capable of forming bone in ectopic formation assays, such as subcutaneous implantation, specific functions likely exist for each of the BMPs. For instance, although BMP7 could be used as a bone morphogenetic protein to form bone ectopically, *BMP7* knockout mice show altered phenotypes in kidney development and the number of digits, but there was no major alteration of bone phenotype (5). This result differs from the embryonic lethal phenotype observed in *BMP2/BMP4* double knockout mice. The data suggest that specific functions may exist for each of the BMPs, although in the case of bone, the total amounts of BMP may be redundant.

Conditional knockout experiments indicate that when BMP activity was knocked out using the *Prx1-Cre* system, bone formation in *BMP2* and *BMP4 Cre* double knockouts could be observed (9). However, the basic patterns of skeletal formation were maintained. Thus, cartilaginous components in the limb bone could be formed almost

Copyright 2006 International Bone and Mineral Society

normally in the absence of BMP2 and BMP4. On the other hand, bone formation *per se* was significantly suppressed, especially in the *BMP2* single knockout mice (9). In addition, there was a major delay in the healing of factures in conditional *BMP2* knockout mice. Thus, these observations reveal a functional role for BMP in adult bones.

Downstream of BMP signaling, there are a number of molecules that are specific to the regulation of osteoblastic function. The most important transcription factors downstream of BMP are Runx2 and Osterix. Deletion of either of these two transcription factors resulted in loss of bone in the transgenic animals (10-17). In addition to the discoveries of BMP function in skeletogenesis, BMP has been found to play roles in many biological events, including morphogenesis (5) and determination of stemness (6). Therefore, BMP is regarded as a central cytokine that regulates not only bone formation but also a number of biological and physiological functions in the living organism. In terms of bone, however, BMP studies have revealed its critical role with respect to the developmental cues in the body (5;7;8). Furthermore, recent studies have shown the importance of BMP in the regulation of adult bone mass. By over-expression of the BMP antagonist Noggin, Canalis *et al*. revealed a reduction in adult bone mass and predicted that BMP is acting to determine adult bone mass (18-20).

Several inhibitory molecules appear to target the function of BMP. Among them is a group of BMP inhibitors that play a role in the determination of adult bone mass. SOST is one such molecule whose expression is related to pathology. SOST is expressed in osteoblastic cells, osteocytes (21) and osteoclasts (21). Although expressed in osteocytes, SOST may be transported through canaliculi in bone to reach osteoblasts on the surface of the bone. Loss-of-function mutations in the *SOST* gene result in the phenotype called van Buchem disease, or sclerosteosis (OMIM 269500) (21), which is characterized by thickening of the bones. When the skull bones become thickened, brain herniation can occur due to the increase in intracranial pressure, leading to sudden death by compression of the brain stem. In the long bones of sclerosteosis patients, the cortex may be thickened, which usually results in fragility of cortical bone due to a reduction in sensitivity for receiving mechanical stress signaling. This paradoxical phenomenon is suspected to be caused by a reduction in mechanical strain when the cortical bones become too thick. SOST is expressed in the developmental period as well (22), around the vertebral body as well as cranial bone. These observations suggest that certain functions of SOST exist during development. Defects in developmental SOST function may be compensated for by an as yet unknown mechanism, since no major phenotype is reported in sclerosteosis patients at birth. Thus, the function of SOST has not yet been fully elucidated and should be pursued further in the future. Nonetheless, SOST is one example where bone formation as well as BMP could be regulated by certain factors in adult bone. Recent analysis of SOST indicated that it could bind to and inhibit the activity of BMP6 and BMP7 (23), although the importance of this phenomenon is not known. It is intriguing that SOST is also expressed in osteoclasts (21). Furthermore, SOST was found to inhibit Wnt3A (24) and to bind to the YWTD-EGF repeat domains of LRP5 and LRP6 thereby blocking the Wnt signaling pathway (25;26). This would be an alternative mechanism of its action on bone mass. It remains to be determined whether SOST acts mainly as an antagonist of BMP or Wnt signaling in adult bone.

Other types of BMP modulators that determine the levels of bone mass in adult mice have been identified. One of these molecules is Tob, which was first identified by Yamamoto *et al.* as a protein that binds to erbB2 (27). Tob family members consist of Tob, Tob2, ANA, and other proteins, all of which contain a Tob homology domain as a motif (27). When Tob molecules are over-expressed in cells, Tob shows inhibitory activity against proliferation (28).

Copyright 2006 International Bone and Mineral Society

BoneKEy-Osteovision. 2006 April;3(4):5-11
http://www.bonekey-ibms.org/cgi/content/full/ibmske;3/4/5
DOI: 10.1138/20060205

Furthermore, BMP signaling is also blocked by Tob over-expression in osteoblastic cultures, based on alkaline phosphatase expression. Conversely, Tob deficiency promotes alkaline phosphatase activity at baseline or in response to BMP treatment. Co-localization of Tob and Smad within nuclei suggests that Tob acts by inhibiting Smad, at least in part by the co-translocation of Smad from the cytosolic to the nuclear compartment (27).

In adult *Tob* knockout mice, bone mass is increased, suggesting that *Tob* regulates bone volume (27). When ovariectomy was carried out in *Tob* knockout mice, the reduction of bone mass was similar between *Tob* knockout and wild type mice (29). However, the baseline level of bone mass was higher in *Tob* knockout mice compared to wild type mice. Therefore, the resulting bone mass level, even after reduction due to ovariectomy in *Tob* knockout mice, was similar to that in wild type sham-operated mice. Time course studies indicated that over time, bone mass levels in *Tob* knockout mice were not reduced further and the final level of bone mass was comparable to sham-operated wild type mice. Thus, the increase in bone mass due to *Tob* deficiency was comparable to the decrease in bone mass due to ovariectomy, *i.e.*, estrogen depletion in these mice. Therefore, at least in animal models, bone mass reduction due to estrogen deficiency could be almost completely compensated for by the absence of *Tob*, since *Tob* deficiency increased bone mass based on the release of inhibition of BMP activity. Based on histomorphometric analysis and bone formation parameters, *Tob* deficiency apparently enhanced the mineral apposition rate (MAR) and bone formation rate (BFR). In animal models of postmenopausal osteoporosis, ovariectomy enhances bone turnover both on the side of bone formation as well as on the side of bone resorption. This defines a high turnover osteoporosis state. In mice deficient in *Tob*, bone resorption occurred, as mentioned, at levels observed in wild type mice. The increase in bone resorption following ovariectomy is coupled to the increase in bone formation in wild type mice. Although *Tob* deficiency had already increased the levels of MAR and BFR by release of the blockage on BMP action, ovariectomy further enhanced BFR and MAR levels, suggesting the presence of a reservoir for bone formation activity in the animals. Such super-induction of bone formation parameters *in vivo* in bone is rarely observed in other circumstances (29;30). On the other hand, bone resorption parameters, such as osteoclast number (OcN/BS) or osteoclast surface (OcS/BS), were similar between the wild type and *Tob* knockout mice. Moreover, the levels of ovariectomy-induced enhancement in bone resorption parameters such as osteoclast number and osteoclast surface were similar between the wild type and *Tob* deficient mice. These observations indicate that *Tob* deficiency specifically affects osteoblastic activity rather than osteoclastic activity.

In order to determine the cellular function of *Tob*, bone marrow cells were prepared from wild type and *Tob* knockout mice, either sham-operated or ovariectomized (29). The bone marrow cells were cultured for three weeks in the presence of ascorbate and β-glycerophosphate to induce bone nodule formation in culture. Similar to the *in vivo* observations, ovariectomy increased bone nodule formation in cultures of bone marrow cells in wild type mice. In *Tob* knockout mice, baseline levels of bone nodule formation were already increased compared to sham-operated wild type mice, and these levels were quite similar to what was observed in cultures of bone marrow cells taken from ovariectomized mice. Interestingly, ovariectomy in *Tob* knockout mice resulted in an exaggerated increase in nodule formation activity *in vitro*. This result is compatible with *in vivo* observations of bone formation parameters in *Tob* knockout mice in terms of the MAR and BFR. With regard to osteoclastic development in the bone marrow cell culture, ovariectomy enhanced osteoclast development in cultures of bone marrow in wild type mice. Sham-operated *Tob* knockout mice showed very similar levels of osteoclast development compared to sham-operated wild type mice. After ovariectomy, *Tob* knockout mice again

Copyright 2006 International Bone and Mineral Society

showed an increase in osteoclast development. However, this increase was quite comparable to what was seen in the wild type mice-derived bone marrow cells. Thus, with regard to the cell culture experiments, the *Tob* target was shown to be cells of the osteoblastic lineage.

Several genes that encode proteins related to osteoblast phenotypes were examined by RT-PCR analysis of RNA taken from the bone marrow of mice that were either sham-operated or ovariectomized (29). Both wild type and *Tob* knockout mice were evaluated in these studies. Compared to sham-operated mice, ovariectomy in wild type mice enhanced expression of Runx2, type I collagen and other osteoblast marker-related genes. After ovariectomy, *Tob*-deficient mice exhibited an exaggerated increase in the expression of Runx2, type I collagen and other osteoblast marker genes.

The expression of osteoclast-related genes, such as *RANKL* and *RANK* or *OPG*, was enhanced by ovariectomy. However, no difference was observed in terms of this increase between the wild type and *Tob* knockout mice. *Tob* deficiency did not alter the function or the response in osteoclasts.

To determine the target sequences of *Tob* deficiency, Smad response elements such as GCCG were used to detect transcription of a luciferase reporter in osteoblastic cells (29). *Tob* deficiency clearly enhanced BMP or Smad-induced luciferase expression, and expression was suppressed by the presence of estrogen. Removal of *Tob* over-expression or removal of the treatment with estrogen indicated additive effects on the increase in transcription levels, suggesting that the targets of *Tob* action and estrogen action converge on Smad responsive sequences. These observations suggested that *Tob* action suppresses bone mass levels in adult mice based, at least in part, on transcriptional events.

Variety in BMP inhibitors would give rise to more variation with regard to the pathogenesis of osteoporosis (20;31). Tob is not the only inhibitory molecule to block BMP and to suppress bone mass levels in adult mice (27-29). Another molecule that has been regarded as a BMP inhibitor and that suppresses bone mass levels in adult mice is the Cas Interactive Zinc finger protein (CIZ) (32;33). This molecule was cloned by Nakamoto and Hirai by using the SH3 domain of p130 Cas as bait in West-Western screening. The same molecule was identified by Bidwell *et al.* (34) as nuclear matrix protein (NMP) 4, which binds the regulatory region of the type I collagen gene. The CIZ molecule was found to colocalize with vinculin in adhesion plagues and has been shown to shuttle between cytosolic and nuclear compartments. Within the nuclear compartment, CIZ modulates expression of MMP genes through its interaction with the sequence C/GAAAAA. When CIZ is over-expressed in MC3T3E1 osteoblastic cells, alkaline phosphatase activity is suppressed (35). Suppression of osteocalcin or Cbfa1 mRNA expression also was observed in response to BMP treatment (35). Furthermore, over-expression of CIZ was shown to suppress mineralized nodule formation induced by the treatment with β-glycerophosphate and ascorbic acid *in vitro*. Thus, at the cellular level, CIZ was found to antagonize BMP activity. When *CIZ* knockout mice were produced, their body morphology was more or less normal, but spermatogenesis was suppressed in these mice (33). Furthermore, analysis of bone indicated that bone mass levels were increased in *CIZ* knockout mice compared to wild type mice (36). Histomorphometric analyses indicated that both the bone formation rate and mineral apposition rate were increased in *CIZ* knockout mice. However, osteoclastic parameters such as osteoclast number and osteoclast surface were not enhanced in these knockout mice. Thus, CIZ appears specifically to suppress bone formation activities in adult mice (35;36).

The levels of messenger RNA encoding Osterix, type I collagen, alkaline phosphatase and osteocalcin were enhanced by the absence of *CIZ* (36). Furthermore, direct BMP injection to calvarial bone increased new bone

Copyright 2006 International Bone and Mineral Society

formation more in *CIZ* knockouts than in the wild type, suggesting that the function of *CIZ* is to block BMP action *in vivo*. Bone marrow ablation was conducted to study injury-related bone formation. The amount of newly formed bone after bone marrow ablation in *CIZ* knockout mice was greater than in wild type mice. These observations in *CIZ* knockout mice suggested that bone mass levels were negatively regulated by endogenous factors (20;37). Intriguingly, CIZ was found to be identical to nuclear matrix protein 4 (34).

Adult bone mass regulation by BMP also has been evaluated using a transgenic mouse system. In these experiments, the BMP antagonist Noggin was expressed specifically in osteoblastic cells by the 2.3 kb type I collagen promoter (18-20). Mice containing type I collagen promoter-regulated Noggin exhibited reduced bone mass (19). Thus, these results indicate that BMP activity in adult bone is important for the maintenance of bone mass throughout adult life. Although simple *Noggin* knockout mice showed developmental defects in joints, the mutation is perinatally lethal (38) and thus, unsuitable to determine effects of Noggin on adult bone mass. These mice had joint spaces that were completely filled with severely disorganized hyperplastic cartilage. It is interesting to note that *Noggin* is highly expressed in cartilage but minimally expressed in bone (22). Therefore, during the development of bone as well as in the maintenance of adult bone mass, Noggin may not be a functional endogenous inhibitor for the determination of adult bone mass, even though transgenic mice indicated a potential function for Noggin in bone. Results from transgenic mouse experiments indicated that disruption of BMP function by Noggin could lead to suppression of bone mass levels, indicating that BMP acts throughout the life of adult mice. Our observation with the *CIZ* knockout mice may indicate that CIZ, instead of Noggin, may play the role of endogenous inhibitor.

Conflict of Interest: The author reports that no conflict of interest exists.

References

1. Riley EH, Lane JM, Urist MR, Lyons KM, Lieberman JR. Bone morphogenetic protein-2: biology and applications. *Clin Orthop Relat Res*. 1996 Mar;324:39-46.

2. Seeherman H, Wozney JM. Delivery of bone morphogenetic proteins for orthopedic tissue regeneration. *Cytokine Growth Factor Rev*. 2005 Jun;16(3):329-45.

3. Miyazono K, Maeda S, Imamura T. BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev*. 2005 Jun;16(3):251-63.

4. Wan M, Cao X. BMP signaling in skeletal development. *Biochem Biophys Res Commun*. 2005 Mar 18;328(3):651-7.

5. Kishigami S, Mishina Y. BMP signaling and early embryonic patterning. *Cytokine Growth Factor Rev*. 2005 Jun;16(3):265-78.

6. Zhang J, Li L. BMP signaling and stem cell regulation. *Dev Biol*. 2005 Aug 1;284(1):1-11.

7. Yoon BS, Lyons KM. Multiple functions of BMPs in chondrogenesis. *J Cell Biochem*. 2004 Sep 1;93(1):93-103.

8. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors*. 2004 Dec;22(4):233-41.

9. Tsuji K, Harfe BD, Cox K, Bouxsein M, Gerstenfeld L, Bandyopadhyay A, Tabin C, Rosen V. Absence of BMP2 results in low bone mineral density, osteoarthritis, spontaneous fracture and impaired fracture healing. *J Bone Miner Res*. 2005 Sep;20(Suppl 1):S8. [Abstract]

10. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada

Copyright 2006 International Bone and Mineral Society

M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell*. 1997 May 30;89(5):755-64.

11. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*. 2002 Jan 11;108(1):17-29.

12. Ito Y, Miyazono K. RUNX transcription factors as key targets of TGF-beta superfamily signaling. *Curr Opin Genet Dev*. 2003 Feb;13(1):43-7.

13. Franceschi RT, Xiao G. Regulation of the osteoblast-specific transcription factor, Runx2: responsiveness to multiple signal transduction pathways. *J Cell Biochem*. 2003 Feb 15;88(3):446-54.

14. Leboy P, Grasso-Knight G, D'Angelo M, Volk SW, Lian JV, Drissi H, Stein GS, Adams SL. Smad-Runx interactions during chondrocyte maturation. *J Bone Joint Surg Am*. 2001;83-A Suppl 1(Pt 1):S15-22.

15. Avsian-Kretchmer O, Hsueh AJ. Comparative genomic analysis of the eight-membered ring cystine knot-containing bone morphogenetic protein antagonists. *Mol Endocrinol*. 2004 Jan;18(1):1-12.

16. Koga T, Matsui Y, Asagiri M, Kodama T, de Crombrugghe B, Nakashima K, Takayanagi H. NFAT and Osterix cooperatively regulate bone formation. *Nat Med*. 2005 Aug;11(8):880-5.

17. Celil AB, Hollinger JO, Campbell PG. Osx transcriptional regulation is mediated by additional pathways to BMP2/Smad signaling. *J Cell Biochem*. 2005 Jun 1;95(3):518-28.

18. Gazzerro E, Pereira RC, Jorgetti V, Olson S, Economides AN, Canalis E. Skeletal overexpression of gremlin impairs bone formation and causes osteopenia. *Endocrinology*. 2005 Feb;146(2):655-65.

19. Devlin RD, Du Z, Pereira RC, Kimble RB, Economides AN, Jorgetti V, Canalis E. Skeletal overexpression of noggin results in osteopenia and reduced bone formation. *Endocrinology*. 2003 May;144(5):1972-8.

20. Canalis E, Economides AN, Gazzerro E. Bone morphogenetic proteins, their antagonists, and the skeleton. *Endocr Rev*. 2003 Apr;24(2):218-35.

21. van Bezooijen RL, Roelen BA, Visser A, van der Wee-Pals L, de Wilt E, Karperien M, Hamersma H, Papapoulos SE, ten Dijke P, Lowik CW. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. *J Exp Med*. 2004 Mar 15;199(6):805-14.

22. Ohyama Y, Nifuji A, Maeda Y, Amagasa T, Noda M. Spaciotemporal association and bone morphogenetic protein regulation of sclerostin and osterix expression during embryonic osteogenesis. *Endocrinology*. 2004 Oct;145(10):4685-92.

23. Kusu N, Laurikkala J, Imanishi M, Usui H, Konishi M, Miyake A, Thesleff I, Itoh N. Sclerostin is a novel secreted osteoclast-derived bone morphogenetic protein antagonist with unique ligand specificity. *J Biol Chem*. 2003 Jun 27;278(26):24113-7.

24. Winkler DG, Sutherland MS, Ojala E, Turcott E, Geoghegan JC, Shpektor D, Skonier JE, Yu C, Latham JA. Sclerostin inhibition of Wnt-3a-induced C3H10T1/2 cell differentiation is indirect and mediated by bone morphogenetic proteins. *J Biol Chem*. 2005 Jan 28;280(4):2498-502.

Copyright 2006 International Bone and Mineral Society

25. Semenov M, Tamai K, He X. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J Biol Chem*. 2005 Jul 22;280(29):26770-5.

26. Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J, Harris SE, Wu D. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J Biol Chem*. 2005 May 20;280(20):19883-7.

27. Yoshida Y, Tanaka S, Umemori H, Minowa O, Usui M, Ikematsu N, Hosoda E, Imamura T, Kuno J, Yamashita T, Miyazono K, Noda M, Noda T, Yamamoto T. Negative regulation of BMP/Smad signaling by Tob in osteoblasts. *Cell*. 2000 Dec 22;103(7):1085-97.

28. Yoshida Y, von Bubnoff A, Ikematsu N, Blitz IL, Tsuzuku JK, Yoshida EH, Umemori H, Miyazono K, Yamamoto T, Cho KW. Tob proteins enhance inhibitory Smad-receptor interactions to repress BMP signaling. *Mech Dev.* 2003 May;120(5):629-37.

29. Usui M, Yoshida Y, Tsuji K, Oikawa K, Miyazono K, Ishikawa I, Yamamoto T, Nifuji A, Noda M. Tob deficiency superenhances osteoblastic activity after ovariectomy to block estrogen deficiency-induced osteoporosis. *Proc Natl Acad Sci U S A*. 2004 Apr 27;101(17):6653-8.

30. Usui M, Yoshida Y, Yamashita T, Tsuji K, Isao I, Yamamoto T, Nifuji A, Noda M. Enhancing effect of Tob deficiency on bone formation is specific to bone morphogenetic protein-induced osteogenesis. *J Bone Miner Res*. 2002 Jun;17(6):1026-33.

31. Lories RJ, Derese I, Luyten FP. Modulation of bone morphogenetic protein signaling inhibits the onset and progression of ankylosing enthesitis. *J Clin Invest*. 2005 Jun;115(6):1571-9.

32. Nakamoto T, Yamagata T, Sakai R, Ogawa S, Honda H, Ueno H, Hirano N, Yazaki Y, Hirai H. CIZ, a zinc finger protein that interacts with p130(cas) and activates the expression of matrix metalloproteinases. *Mol Cell Biol*. 2000 Mar;20(5):1649-58.

33. Nakamoto T, Shiratsuchi A, Oda H, Inoue K, Matsumura T, Ichikawa M, Saito T, Seo S, Maki K, Asai T, Suzuki T, Hangaishi A, Yamagata T, Aizawa S, Noda M, Nakanishi Y, Hirai H. Impaired spermatogenesis and male fertility defects in CIZ/Nmp4-disrupted mice. *Genes Cells*. 2004 Jun;9(6):575-89.

34. Shah R, Alvarez M, Jones DR, Torrungruang K, Watt AJ, Selvamurugan N, Partridge NC, Quinn CO, Pavalko FM, Rhodes SJ, Bidwell JP. Nmp4/CIZ regulation of matrix metalloproteinase 13 (MMP-13) response to parathyroid hormone in osteoblasts. *Am J Physiol Endocrinol Metab*. 2004 Aug;287(2):E289-96.

35. Shen ZJ, Nakamoto T, Tsuji K, Nifuji A, Miyazono K, Komori T, Hirai H, Noda M. Negative regulation of bone morphogenetic protein/Smad signaling by Cas-interacting zinc finger protein in osteoblasts. *J Biol Chem*. 2002 Aug 16;277(33):29840-6.

36. Morinobu M, Nakamoto T, Hino K, Tsuji K, Shen ZJ, Nakashima K, Nifuji A, Yamamoto H, Hirai H, Noda M. The nucleocytoplasmic shuttling protein CIZ reduces adult bone mass by inhibiting bone morphogenetic protein-induced bone formation. *J Exp Med*. 2005 Mar 21;201(6):961-70.

37. Krane SM. Identifying genes that regulate bone remodeling as potential therapeutic targets. *J Exp Med*. 2005 Mar 21;201(6):841-3.

38. Brunet LJ, McMahon JA, McMahon AP, Harland RM. Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton. *Science*. 1998 May 29;280(5368):1455-7.

Copyright 2006 International Bone and Mineral Society