Bone morphogenetic proteins (BMPs) function during various aspects of embryonic development including skeletogenesis. However, their biological functions after birth are less understood. To investigate the role of BMPRIA during bone remodeling, we generated a postnatal osteoblast-specific disruption of Bmpr1a that encodes the type IA receptor for BMP in mice. Mutant mice were smaller than controls up to 6 months after birth. Irregular calcification and low bone mass were observed, but there were normal numbers of osteoblasts. The ability of the mutant osteoblasts to form mineralized nodules in culture was severely reduced. Interestingly, bone mass was increased in aged mutant mice due to reduced bone resorption evidenced by reduced bone turnover. The mutant mice lost more bone after ovariectomy likely resulting from decreased osteoclast-mediated bone resorption. In organ culture of bones from aged mice, ablation of the Bmpr1a gene by adenoviral Cre recombinase abolished the stimulatory effects of BMP4 on the expression of lysosomal enzymes essential for osteoclastic bone resorption. These results demonstrate essential and age-dependent roles for BMP signaling mediated by BMPRIA (a type IA receptor for BMP) in osteoblasts for bone remodeling.

Bone formation is a well characterized process; however, little is known about the molecular mechanisms that regulate bone remodeling, the physiological process through which bone mass is maintained constant. Remodeling consists of two distinct phases: initial bone resorption by the osteoclasts, followed by de novo bone formation by the osteoblasts (1). Differentiated osteoblasts are the only cells responsible for bone formation. Bone formation is thought to be regulated by hormones and by locally acting growth factors (2). Bone morphogenetic proteins (BMPs) are secreted molecules and members of transforming growth factor-β superfamily (3, 4). They were discovered by their ectopic bone formation activity when implanted locally in soft tissues (5). Over the past decade, the phenotypes of mice with mutations in genes coding for this group of proteins and their receptors unveiled the essential roles for BMPs in wide variety of developmental processes, including skeletal development and patterning (6–9). However, despite its powerful ability to induce ectopic osteogenesis, the essential role of BMPs in bone formation and bone metabolism in the adult skeleton has not been established (10) because of embryonic lethality resulting from mutations of genes encoding the most potent BMPs for bone formation, BMP2 and BMP4, and their receptors (11–13).

We previously generated a null allele for Bmpr1a that encodes a type IA receptor for BMP (BMPRIA or ALK3). Mice heterozygous for this null allele died by embryonic day 11.5 (12) without mesodermal formation (13). Bmpr1a is expressed in most tissues throughout development and after birth (13, 14). Expression of a dominant-negative form of BMPRIA in a cultured cell line or chick limb buds suggests that signaling through this receptor regulates apoptosis and adipocyte differentiation (15, 16). Overexpression of a constitutive-active form of BMPRIA in chicken limb buds suggests that signaling through this receptor also can regulate chondrocyte differentiation (17). However, the essential role of BMPRIA in bone formation and bone metabolism in the adult skeleton is not known. To investigate the role of BMPRIA signaling at later stages of development, we generated a conditional null allele of Bmpr1a using the Cre/lox system (18, 19). Because Bmpr1a is expressed in osteoblasts (20), we designed a postnatal, differentially osteoblast-specific disruption of Bmpr1a to elucidate the requirement of BMP signaling for bone formation (21).

**MATERIALS AND METHODS**

**Mice**—The generation of Bmpr1a conditional null mice was reported elsewhere (19). Briefly, one loxP site was placed in intron 1, and two others were placed in intron 2 flanking a PGK-neo cassette (fx allele). After germ line transmission, mice heterozygous for the fx allele were mated with CMV-Cre transgenic mice to remove the neo cassette by Cre-dependent recombination in vivo (fx allele). Both the fx allele and the fx allele behaved as wild type, indicating that the presence of the PGK-neo cassette or the loxP sites in the Bmpr1a locus did not reduce Bmpr1a activity (19). For the studies reported here we used both the fx and fx alleles. Mice heterozygous for Bmpr1a null allele (+/−) were bred to O2-2-Cre transgenic mice (21) to generate Bmpr1a(+/−)/O2-2-Cre(+/−) mice.

The abbreviations used are: BMP, bone morphogenetic protein; BMPRIA, a type IA receptor for BMP; BMD, bone mineral density; TRAP, tartrate-resistant acid phosphatase; Mmp9, matrix metalloproteinase-9 gene; Ctsk, cathepsin K gene; BTVT, bone volume per total volume; BFR/Bs, bone formation rate per total bone surface; OVX, ovariectomy; FAM, 6-carboxyfluorescein.

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BMP Signaling Regulates Osteoblast Function

RESULTS

Disruption of the Type I A Receptor for BMPs in a Differentiated Osteoblast-specific manner—Osteocalcin2 (Og2) is specifically expressed in differentiated osteoblasts only after birth (24, 25). To direct Cre recombinase in postnatal differentiated osteoblasts, we used transgenic mice that carry a 1.3-kb mouse Og2 upstream region ligated to the Cre recombinase gene (21). Osteoblast-specific Cre activity was examined by crosses with CAT-lacZ mice that report Cre activity by β-galactosidase expression (26). In Og2-Cre, CAT-lacZ double heterozygotes, all bones stained positively for β-galactosidase activity (Fig. 1A), resembling the β-galactosidase pattern of Og2-lacZ transgenic mice (27). β-Galactosidase activity was observed only in the osteoblasts on trabecular bone near the growth plates and on cortical bone surfaces (Fig. 1, B, C, E, and F). No β-galactosidase activity was detected in the soft tissues, bone marrow, or chondrocytes, indicating that expression of Cre in Og2-Cre mice was highly specific for osteoblasts.

Pups transheterozygous for the conditional null and null alleles of Bmpr1a, and hemizygous for the Og2-Cre transgene, were recovered after birth. As shown in Fig. 2A, they were smaller than their control littermates at 2 months of age. Smaller body size was recognizable as early as 3 weeks of age and became more prominent at 6 or 12 weeks (Fig. 2B). Their body weight remained below normal for up to 6 months. Because the proportion of the mature osteoblasts was too small for...
detection of the Cre-dependent recombination by Southern blot, floxed and recombined alleles were differentiated by PCR. All of the mice that carried the Og2-Cre transgene showed at least one recombined allele that had deleted exon 2 (Fig. 2C).

Reduced Osteoblast Function in the Mutant Mice—Contact x-ray photography of 3-month-old mice showed no overt changes in bone shape compared with controls (Fig. 3A). However, irregular calcification was found, most prominently in femurs (Fig. 3, B and C, arrow). Histological analysis showed decreased bone trabeculae (Fig. 3, D and E) with no growth plate abnormalities.

Osteopontin and osteocalcin were expressed normally in the mutant bones (data not shown). To investigate the cause of the altered bone formation, we performed a morphometric analysis of undecalcified histological sections (23), which revealed a decrease in bone volume in the mutant mice that was about half of that in their control littermates (Fig. 3F). Bone formation rate was reduced in mutants in comparison with controls (Fig. 3G). In addition, the numbers of osteoblasts and osteoclasts in tibia and spine did not differ significantly between mutants and their controls (data not shown).

Fig. 2. Osteoblast-specific disruption of Bmpr1a causes reduced growth. A, 2-month-old females (top) and males (bottom). Three pairs of mice from the same litter are shown (Bmpr1a fn/(−), Og2-Cre mice should lose BMPR1A in a differentiated osteoblast-specific manner). Bar, 1 cm. B, body weight of female (top) and male (bottom) littermates. Among four different genotypes, only Bmpr1a fn/(−), Og2-Cre showed reduced body weight (shaded columns). The number of animals is shown above each bar. p < 0.005. C, PCR detection of Cre-dependent recombination. DNA extracted from bone was amplified with allele-specific PCR primers shown. All mice analyzed here were heterozygous for the fn allele. All of the mice that carried the Og2-Cre transgene showed the deletion of exon2. D10 and D8 are ES cell clones heterozygous for Δexon2-neo and Δexon2 alleles, respectively, and D10 is mosaic for fn and Δexon2-neo (19).
Consistent with the morphometric analysis, in *in vitro* culture of bone marrow cells from mutant and control mice, the osteoblastic colonies derived from Bmpr1a*fx/−*Og2-Cre(+) mice were smaller than those derived from control cells and stained poorly for mineral deposition (Fig. 4, A and B). Fewer alkaline phosphatase-positive colonies and von Kossa-positive colonies were observed in the culture derived from bone marrow cells in mutant mice (Fig. 4C). Taken together, the *in vitro* and *in vivo* analyses suggest that osteoblast-specific Bmpr1a disruption leads to less osteoblast function and loss of bone formation, resulting in decreased bone remodeling and decreased body size in mutant mice.

Reduced Osteoclast Activity in Aged Mutant Mice—Although younger mutant mice (up to 6 months) weighed less than controls (Fig. 2), the difference became smaller as they got older (data not shown). Intriguingly, BMD in 10-month-old mutant mice was significantly higher than that of controls (Fig. 5A, WT/Sham and KO/Sham). Because there were no significant differences in BMD observed at 3 months of age (data not shown), these findings suggested that loss of BMP signaling in osteoblasts might lead to down-regulation of osteoclast function as the mice aged. Histomorphometric analysis of femurs of 10-month-old mice confirmed the higher bone volume (BV/TV (bone volume per total volume)) in mutant mice compared with controls (Fig. 5F, WT/Sham and KO/Sham). Dynamic histomorphometric analysis with double calcein labeling documented that bone turnover (BFR/BS (bone formation rate per total bone surface)) is decreased in mutant mice (Fig. 5G, WT/Sham and KO/Sham), indicating that increased bone mass in mutant mice is due to decreased bone resorption. These results suggest that, in aged animals, loss of BMP signaling via BMPRIA in osteoblasts may lead to suppression of osteoclast function and bone resorption. To gain further insights into the role of BMPRIA and BMP signaling in adult bone metabolism, we evaluated the effects of ovariectomy (OVX) that activates bone turnover and osteoclastic bone resorption, leading to bone loss. Interestingly, the loss of BMD in the OVX group, compared with the sham operated group, was more significant in mutant mice (Fig. 5A). Histological observations agreed with the BMD findings (Fig. 5, B–E). More trabecular bone is observed in the distal femur of the mutant mice compared with the controls, and in both mutant and control mice, trabecular bone was severely reduced by OVX. Quantitative histomorphometric analysis confirmed these findings by documenting the significant reduction of bone volume (BV/TV) in OVX groups to Sham-operated groups with more significant effects of OVX observed in mutant mice (Fig. 5F). More significant bone loss induced by OVX in mutant mice likely results from the decreased osteoblast function, observed *in vivo* and *in vitro* analyses in young animals, that became evident in high bone turnover induced by OVX. Taken together, the increased bone mass and the greater degree of bone loss...
enzymes secreted from osteoclasts, in calvaria were all increased with BMP4 treatment. These results are consistent with the role of the BMPRIA signaling pathway in osteoblastic bone resorption postulated by the reduced bone resorption observed in mutant mice. In contrast, in calvaria following treatment with Cre recombinase that inactivates Bmpr1a, BMP4 treatment showed no effects on the levels of expression of TRAP, Mmp9, and Ctsk (Fig. 6, C–E). Basal levels of these enzymes were also reduced by inactivation of Bmpr1a. Interestingly, the expression of calcitonin receptor, a marker of osteoclast, was not induced by BMP4 nor regulated by treatment with Cre recombinase (data not shown), suggesting the specific effects of the BMP-BMPRIA signaling pathway in expression of lysosomal enzymes essential for osteoclastic bone resorption. Expression of RANKL that is expressed in osteoclasts to support osteoclast function was not changed by Cre treatment (data not shown). Similar results were observed by organ culture of tibia from the same animals (data not shown). Analysis of marker genes for osteoblasts, such as type I collagen or osteocalcin, did not show significant changes under these experimental conditions (data not shown).

**DISCUSSION**

Our results show that signaling through the BMP type IA receptor in differentiated osteoblasts plays an important role in bone formation when mice are relatively young (>6 months of age). The mutant mice showed reduced body weight, lower bone volume, and reduced bone formation rate. This signal is apparently not be essential for osteoblast differentiation or proliferation but is important for the production of bone matrix by differentiated osteoblasts. Intriguingly, the differentiated osteoblast-specific disruption of the BMP type IA receptor leads to an opposite phenotype when mice become older (at 10 months of age). Bone volume in the mutant mice was higher than that of control littermates even though bone formation rate was still lower in the mutants. The mutant mice that were ovariectomized showed the same BMD and bone volume as the controls that were ovariectomized, suggesting that BMP signaling through the type IA receptor in the differentiated osteoblasts may be important for regulating osteoclast activity. This hypothesis is supported by the induction of lysosomal enzymes essential for osteoclastic bone resorption by BMP4 in calvarial organ culture that is abolished following inactivation of Bmpr1a.

It has been shown that alteration of BMP signaling during embryogenesis affects skeletalgenesis (6–9, 28–31). Gene disruption of the type IB receptor for BMPs (Bmpr1b) shows defects in the proximal and middle phalanges (32, 33), which resembles that in Gdf5 mutant mice, brachypodism (7). A transgenic mouse line that expresses a dominant-negative form of Bmpr1b in an osteoblast-specific manner shows more severe abnormalities in the skeletal system than Bmpr1b-deficient mice such as reduced BMD, bone volume, and bone formation rate up to 3 months after birth (34). A transgenic mouse line that expresses Noggin, one of the BMP antagonists, in a differentiated osteoblast-specific manner shows reduced bone formation up to 6 months after birth (35). Recently, linkage of osteoporosis to chromosome 20p12 where BMP2 resides was demonstrated in a Danish population (36). These results suggest that BMP signaling in the osteoblasts plays important roles in bone formation during younger stages of life, consistent to the results presented here.

It has been reported that osteoblast play essential roles in supporting the generation and activity of osteoclasts that are critical for bone resorption (37). The stimulatory effects of BMP4 in lysosomal enzymes expressed in osteoclasts in calvarial organ culture is in agreement with previous reports docu-
menting the stimulatory effects of BMPs on osteoclast function in vitro (38–42). BMPs may act directly on osteoclasts (41) or alternatively via osteoblasts (40) because both types of cells express receptors for BMPs. Although we cannot exclude the possible role for Cre-dependent inactivation of Bmpr1a in osteoclasts in the loss of BMP induction of lysosomal enzymes in organ culture, these results along with in vivo results suggest that BMP signaling in osteoblasts controls bone resorption by supporting osteoclast function in aged mice. Our results suggest that BMP functions in differentiated osteoblasts are altered in an age-dependent manner, initially for bone formation at younger ages, then more predominantly for supporting osteoclast function as animals age.

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REFERENCES
1. Ducy, P., Schinke, T., and Karsenty, G. (2000) Science 289, 1501–1504
2. Takeda, S., Elefteriou, F., and Karsenty, G. (2003) Annu. Rev. Nutr. 23, 403–411
3. Hogan, B. L. M. (1996) Genes Dev. 10, 1580–1594
4. Mishina, Y. (2003) Front. Biosci. 8, d855–d869
5. Urst, M. R. (1965) Science 150, 893–899
6. Kingsley, D. M., Bland, A. E., Grubber, J. M., Marker, P. C., Russell, L. B., Copeland, N. G., and Jenkins, N. A. (1992) Cell 71, 399–410
7. Storm, E. E., Huynh, T. V., Copeland, N. G., Jenkins, N. A., Kingsley, D. M., and Lee, S. J. (1994) Nature 368, 639–643
8. Luo, G., Hofmann, C., Bronzek, A. L., Sobocki, M., Bradley, A., and Karsenty, G. (1995) Genes Dev. 9, 2886–2890
9. Dudley, A. T., Lyons, K. M., and Robertson, E. J. (1995) Genes Dev. 9, 2795–2807
10. Karsenty, G., and Wagner, E. F. (2002) Dev. Cell 2, 389–406
11. Winnier, G., Blessing, M., Labosky, P. A., and Hogan, B. L. M. (1995) Genes Dev. 9, 2105–2116
12. Zhang, H., and Bradley, A. (1996) Development (Camb.) 122, 2977–2986
13. Mishina, Y., Suzuki, A., Ueno, N., and Behringer, R. R. (1995) Genes Dev. 9, 3027–3037
14. Dewulf, N., Verschueren, K., Lonny, O., Moren, A., Grimsby, S., Vande Spiegle, K., Miyazono, K., Huylenbroeck, D., and Ten Dijke, P. (1995) Endocrinology 136, 2852–2863
15. Yokouchi, Y., Sakiyama, J., Kameda, T., Iba, H., Suzuki, A., Ueno, N., and Kurosawa, A. (1996) Development (Camb.) 122, 3732–3734
16. Chen, D., Ji, X., Harris, M. A., Feng, J. Q., Karsenty, G., Celeste, A. J., Rosen, V., Mundy, G. R., and Harris, S. E. (1997) J. Biol. Chem. 272, 2191–2203
17. Zou, H., Wieser, R., Massague, J., and Niswander, L. (1997) Genes Dev. 11, 2191–2203
18. Nagy, A. (2000) Genesis 26, 99–109
19. Mishina, Y., Hanks, M. C., Miura, S., Tallquist, M. D., and Behringer, R. R. (2002) Genesis 32, 69–72
20. Ishidou, Y., Kitajuma, I., Obana, H., Maruyama, I., Murata, F., Imanura, T., Yamada, N., ten Dijke, P., Miyazono, K., and Sakou, T. (1995) J. Bone Miner. Res. 10, 1651–1659
21. Daquain, R., Starbuick, M., Schinke, T., and Karsenty, G. (2002) Dev. Dyn. 224, 245–251
22. Weinstein, R. S., Jilka, R. L., Parfitt, A. M., and Manolagas, S. C. (1997) Endocrinology 138, 4015–4021
23. Parfitt, A. M., Dremlner, M. K., Glorieux, F. H., Kanis, J. A., Malluche, H., Meunier, P. J., Ott, S. M., and Recker, R. R. (1987) J. Bone Miner. Res. 2, 595–610
24. Ducy, P., and Karsenty, G. (1995) Mol. Cell. Biol. 15, 1858–1869
25. Desbois, C., Hugue, B. A., and Karsenty, G. (1994) J. Biol. Chem. 269, 1183–1190
26. Araki, K., Araki, M., Miyazaki, J., and Vassalli, P. (1995) Proc. Natl. Acad. Sci. USA 92, 899–903

Fig. 6. Effect of ablation of Bmpr1a on expression of lysosomal enzymes essential for osteoclastic bone resorption. Calvarias were removed from aged mice that were homozygous for floxed Bmpr1a and then cultured with or without recombinant Adenovirus that expressed Cre for 7 days. Subsequently, calvarias were cultured with or without 100 ng/ml BMP4, then the RNA was extracted. Gene expression was measured by real time PCR. A, reduction of a wild-type transcript from Bmpr1a (*, p < 0.0015). B, emergence of a transcript from Cre recombinase allele of Bmpr1a (*, p < 0.0001). C–E, levels of lysosomal enzymes expressed in osteoclasts. Up-regulation of TRAP, Mmp9, and Ctsk with BMP4 (*, p < 0.02) were abolished by treatment with Cre recombinase (**, p < 0.003). The levels of expression of each gene are presented as fold differences relative to the levels expression of each gene in the control group.
27. Frendo, J. L., Xiao, G., Fuchs, S., Franceschi, R. T., Karsenty, G., and Ducy, P. (1998) J. Biol. Chem. 273, 30509–30516
28. Solloway, M. J., Dudley, A. T., Bikoff, E. K., Lyons, K. M., Hogan, B. L., and Robertson, E. J. (1998) Dev. Genet. 22, 321–339
29. Katagiri, T., Boorla, S., Frendo, J. L., Hogan, B. L., and Karsenty, G. (1998) Dev. Genet. 22, 340–348
30. Thomas, J. T., Lin, K., Nandedkar, M., Camargo, M., Cevenka, J., and Luyten, F. P. (1996) Nat. Genet. 12, 315–317
31. Thomas, J. T., Kilpatrick, M. W., Lin, K., Erlacher, L., Lembessis, P., Costa, T., Tsipouras, P., and Luyten, F. P. (1997) Nat. Genet. 17, 58–64
32. Yi, S. E., Daluiski, A., Pederson, R., Rosen, V., and Lyons, K. M. (2000) Development (Camb.) 127, 621–630
33. Baur, S. T., Mai, J. J., and Dymecki, S. M. (2000) Development (Camb.) 127, 605–619
34. Zhao, M., Harris, S. E., Horn, D., Geng, Z., Nishimura, R., Mundy, G. R., and Chen, D. (2002) J. Cell Biol. 157, 1049–1060
35. Devlin, R. D., Du, Z., Pereira, R. C., Kiumb, R. B., Economides, A. N., Jorgetti, V., and Canalis, E. (2003) Endocrinology 144, 1972–1978
36. Styrkarsdottir, U., Cazier, J.-B., Kong, A., Rodifson, O., Larsen, H., Bjarnadottir, E., Johannsdottir, V. D., Sigurdardottir, M. S., Bagger, Y., Christiansen, C., Reynolds, J. I., Grant, S. F. A., Jonasson, K., Frigge, M. L., Gulcher, J. R., Sigurdsson, G., and Stefansson, K. (2003) PLoS Biol. 1, E69
37. Takahashi, N., Akats, T., Udagawa, N., Sasaki, T., Yamaguchi, A., Moseley, J. M., Martin, T. J., and Suda, T. (1998) Endocrinology 139, 2600–2602
38. Hentunen, T. A., Lakkakorpi, P. T., Tuukkanen, J., Lehenkari, P. P., Samath, T. K., and Vaananen, H. K. (1995) Biochem. Biophys. Res. Commun. 209, 433–443
39. Koide, M., Murase, Y., Yamato, K., Noguchi, T., Okahashi, N., and Nishihara, T. (1999) Biochem. Biophys. Res. Commun. 259, 97–102
40. Abe, E., Yamamoto, M., Taguchi, Y., Lecka-Czernik, B., O’Brien, C. A., Economides, A. N., Stahl, N., Jilka, R. L., and Manolagas, S. C. (2000) J. Bone Miner. Res. 15, 663–673
41. Kaneko, H., Arakawa, T., Mano, H., Kaneda, T., Ogasawara, A., Nakagawa, M., Toyama, Y., Yabe, Y., Kumezawa, M., and Hakeda, Y. (2000) Bone 27, 479–486
42. Itoh, K., Udagawa, N., Katagiri, T., Iemura, S., Ueno, N., Yasuda, H., Higashio, K., Quin, J. M., Gillespie, M. T., Martin, T. J., Suda, T., and Takahashi, N. (2001) Endocrinology 142, 3656–3662
43. Traianedes, K., Dallas, M. R., Garrett, J. R., Mundy, G. R., and Bonewitt, L. F. (1998) Endocrinology 139, 3178–3184
