Soluble transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP)-binding proteins are widely distributed in mammalian tissues and control cytokine access to membrane signaling receptors. The serum and bone-resident glycoprotein α2-HS-glycoprotein/fetuin (ASHG) binds to TGF-β/BMP cytokines and blocks TGF-β1 binding to cell surface receptors. Therefore, we examined bone growth and remodeling phenotypes in ASHG-deficient mice. The skeletal structure of Ahsg−/− mice appeared normal at birth, but abnormalities were observed in adult Ahsg−/− mice. Maturation of growth plate chondrocytes was impaired, and femurs lengthened more slowly between 3 and 18 months of age in Ahsg−/− mice. However, bone formation was increased in Ahsg−/− mice as indicated by greater cortical thickness, accelerated trabecular bone remodeling, and increased osteoblast numbers on bone surfaces. The normal age-related increase in cortical thickness and bone mineral density was accelerated in Ahsg−/− mice and was associated with increased energy required to fracture. Bone formation in response to implanted BMP cytokine extended further from the implant in Ahsg−/− compared with Ahsg+/+ mice, confirming the interaction between ASHG and TGF-β/BMP cytokines in vivo. Our results demonstrate that ASHG blocks TGF-β-dependent signaling in osteoblastic cells, and mice lacking ASHG display growth plate defects, increased bone formation with age, and enhanced cytokine-dependent osteogenesis.

ASHG1 or fetuin is a liver secretory glycoprotein found at high levels in serum and mineralized bone (1, 2). The human AHSG gene is located on chromosome 3q27, and two common allelic forms have been identified that correlate with different bone phenotypes (3). Homozygosity for AHSG+/+ allele is associated with shorter stature and reduced bone quality measured by calcaneal broadband ultrasound (4). Further interest stems from the observations that elevated serum AHSG correlates with a common form of mild osteogenesis imperfecta (5), whereas depressed levels are observed in Fugit’s disease, a condition characterized by increased bone turnover (6). AHSG is also a reverse acute-phase reactant, as serum levels decline by 20–30% during acute inflammation (7).

ASHG protein has two cystatin-like domains with a characteristic arrangement of disulfide loops (8), a calcium phosphate-binding site near the N terminus (9), and a TGF-β cytokine-binding motif (10). The latter is a disulfide-looped sequence in the N-terminal cystatin domain from Cys-96 to Cys-114 (human sequence) which shares homology with the extracellular domain of TGF-β receptor type II (TβRII) (Cys-84 to Cys-101). In surface plasmon resonance assays, these peptides bind to TGF-β and BMP cytokines with specificity characteristic of native ASHG and TβRII, suggesting they are the major cytokine-binding domains in each glycoprotein (10). AHSG binds to TGF-β cytokines with KD values ranging from 0.03 to 2.0 μM for BMP-2, BMP-4, BMP-6, TGF-β1, and TGF-β2 in rank order. Furthermore, ASHG blocked TGF-β1 and BMP-2 anti-proliferative and osteogenic activities in cell culture (10). Although the affinity of these interactions is modest, the serum concentration of ASHG is ~12 μM, and based on mass action, ASHG would be expected to influence cytokine availability in vivo. The on and off rates for ASHG-cytokine binding are slow (10), a property characteristic of other cytokine antagonists (11). Endogenous TGF-β1 in rat bone marrow cell cultures is required for differentiation, but at ≥10 pM TGF-β1 completely inhibits mineralization. Osteogenesis was observed to be maximal at ~1 pM TGF-β1, and as might be expected, addition of either ASHG or neutralizing anti-TGF-β1 antibodies increased the required TGF-β1. Therefore, osteogenesis depends on a molar ratio of ASHG to cytokine, suggesting that ASHG may establish morphogenic fields for osteo-induction and thereby control bone remodeling. Osteogenesis in rat bone marrow cell cultures was optimal at ~1/300,000 molar ratio of TGF-β1 to ASHG which reflects the relative physiological levels and activities of these proteins (12).

Spatially distinct sources of cytokine and antagonist are known to establish gradients of cytokine activity and thereby control regional differentiation in the early embryo (13, 14). For example, noggin and chordin establish a BMP-4 gradient in the gastrula stage vertebrate embryo (15). Noggin is also expressed...
in condensing cartilage, and noggin-deficient embryos display abnormal growth plates and joints (16). Other TGF-β cytokine-binding proteins include serum aα-macroglobulin (17), soluble betaglycan (18), matrix proteoglycans, decorin, biglycan, and fibromodulin (19–21). Biglycan-deficient mice display an age-dependent low bone mass (22), demonstrating that matrix-localized cytokine-binding proteins can influence bone homeostasis.

Skeletogenesis precedes expression and accumulation of ASHG in mineralizing bone, and indeed Ahsg<sup>−/−</sup> mice display no obvious anatomical abnormalities at birth (23). However, bone is continuously remodeled during adult life, a process whereby osteoclasts degrade the bone and release cytokines, in turn stimulating osteoblast recruitment from bone marrow to restore the bone. It is possible that bone and serum-derived ASHG bind the released cytokines near the remodeling surface and regulate osteogenic activity. Here we have examined femur histology and geometry, as well as bone structural and dynamic properties in young and old bone mass (22), demonstrating that matrix-localized cytokine-binding proteins can influence bone homeostasis.

**EXPERIMENTAL PROCEDURES**

**ASHG Mutant Mice**—ASHG-deficient mice were generated by targeted gene mutation in embryonic cells, which removed the entire coding region of the gene as described previously (23). An equal distribution of male and female mice with Ahsg<sup>−/−</sup>, Ahsg<sup>−/+</sup>, and Ahsg<sup>+/+</sup> genotypes on a 129sv × C57BL/6 background were generated for the analysis, and the data from both sexes were averaged.

**Immunohistochemistry**—Dissected femurs were fixed in 10% formalin for 7 days, decalcified in 10% formic acid, then dehydrated through a series of ethanol (20–100%) and xylene solutions, and finally embedded in paraffin (Paraplast X-Tra). Blocks were softened with mollifex (BDH) and then sectioned. The sections were deparaffinized, hydrated through xylene and ethanol, incubated with a 1/500 dilution of rabbit anti-mouse ASHG antibody in 1.5% serum in phosphate-buffered saline, washed, then incubated with 1/5000 dilution of biotinylated antimouse antibody (BDH) and then sectioned. The sections were deparaffinized, hydrated through xylene and ethanol, incubated with a 1/500 dilution of rabbit anti-mouse ASHG antibody in 1.5% serum in phosphate-buffered saline, washed, then incubated with 1/5000 dilution of biotinylated anti-rabbit antibody, and developed with the horseradish peroxidase substrate 3,3’-diaminobenzidine tetrahydrochloride (Vector Laboratories). The slides were counterstained with hematoxylin.

**Bone Histomorphometry and Dynamic Properties**—Three-month-old mice were injected in the tail vein with 10 mg of tetracycline/kg at 10 and 3 days prior to sacrifice. The femurs were dissected and cut coronally, and both halves were fixed in 70% ethanol, dehydrated through increasing strengths of acetone, infiltrated in increasing strengths of paraffin (Paraplast X-Tra). Blocks were softened with mollifex (BDH) and then sectioned. The sections were deparaffinized, hydrated through xylene and ethanol, incubated with a 1/500 dilution of rabbit anti-mouse ASHG antibody in 1.5% serum in phosphate-buffered saline, washed, then incubated with 1/5000 dilution of biotinylated anti-rabbit antibody, and developed with the horseradish peroxidase substrate 3,3’-diaminobenzidine tetrahydrochloride (Vector Laboratories). The slides were counterstained with hematoxylin.

**Mechanical Testing and Dual Energy X-ray Absorptiometry**—Destructive three-point bending was performed on the right femurs of
mice using a screw-driven mechanical testing machine (Instron model 1011, Canton, MA). Each bone was placed on two supports spaced 6.7 mm apart, and a load was applied to the bone midway between the supports at a deformation rate of 1 mm/min. From the load displacement curve, the maximum load and maximum displacement were measured, and the stiffness was determined from a linear regression of the initial portion of the curve. The length, diameter, and cortical thickness of the bones were determined using digital calipers. Dual Energy x-ray absorptiometry (Pixi Mus, Lunar Corp., Madison, WI) was used to measure bone mineral content, bone area, and bone mineral density (BMD) of femurs.

RESULTS

Osteogenesis Induction—Femoral bones were removed under aseptic conditions from adult male Wistar rats (120 g), cleaned of adherent soft tissues, and washed extensively in antibiotics. The distal ends were removed, and the marrow contents were flushed out with 10 ml of culture medium. The cells were dispersed by repeated passage through a 20-gauge needle and incubated in α-minimum Eagle’s medium supplemented with 15% fetal bovine serum, ascorbic acid (50 μg/ml), antibiotics (100 μg/ml penicillin G, 50 μg/ml gentamicin, 0.3 μg/ml fungizone), 10 mM β-glycerophosphate, 10−8 M dexamethasone, and vitamin C. Following 6 days of culture, the cells were re-plated at a density of 1 × 106 cells/mm2 in 96-well plates, and grown for another 12–14 days without dexamethasone and with 10 nm recombinant BMP-2. At the end of culture, the cells were fixed with 10% buffered formalin and stained for calcium with Alizarin Red-s to identify mineralized bone matrix, as described previously (32).

Fig. 3. Geometric and structural properties of femurs in Ahsg+/+, Ahsg−/−, and Ahsg−/- mice. The length (A), cortical thickness (B), bone mineral content (C), bone mineral density (D), maximum load (failure) (E), and energy (F) were measured in Ahsg−/−, Ahsg−/-, and Ahsg−/- mice at 3–4 and 12–18 months. The energy and load were determined by three-point bending of femurs. Results are the mean ± S.E. of 12 mice per genotype, and *, p < 0.05 versus Ahsg−/− mice.

Growth Plate Defects and Reduced Femur Length in Ahsg−/- Mice—Serum ASHG was reduced by ~50% in Ahsg−/- and absent in the Ahsg−/- mice as reported previously (23). ASHG protein was concentrated in the mineralized regions of bones (2) from Ahsg+/+ and Ahsg−/- mice (Fig. 1, A and B) and was particularly concentrated in bone surrounding the growth plate (Fig. 1D). The density of ASHG protein displayed heterogeneity in trabecular bone, possibly due to variations in when the bone was last remodeled (Fig. 1E). ASHG protein was more concentrated in bone surrounding osteocytes (Fig. 1F), cells that are encased in bone and respond to mechanical stress by secreting paracrine factors that stimulate bone remodeling (33).
Endochondral ossification by maturing chondrocytes occurs at the proximal aspect of the epiphyseal growth plate and drives longitudinal bone growth. Accordingly, the deficiency in growth plate chondrocyte maturation was associated with reduced longitudinal bone growth in Ahsg<sup>−/−</sup> mice. Femur length in Ahsg<sup>−/−</sup> mice compared with wild type age-matched mice was reduced by 9% at 3–4 months and by 14.7% at 12–18 months (Fig. 3A). However, femoral cortical thickness was significantly increased in the Ahsg<sup>−/−</sup> mice at both 3–4 and 12–18 months of age, indicating an increase in osteoblastic activity relative to osteoclastic activity in Ahsg<sup>−/−</sup> mice (Fig. 3B). Heterozygous mice were not significantly different from wild type for these measurements.

**BMD and Strength Increase with Age in Ahsg<sup>−/−</sup> Mice**—Changes in cortical thickness are expected to alter the mechanical and structural properties of bone. Bone mineral content and bone area were determined using dual energy x-ray absorptiometry analysis of mice at 3–4 months and at 12–18 months of age. The Ahsg<sup>−/−</sup> mice displayed no significant change in bone mineral content, but BMD, which is bone mineral content normalized to bone area, was increased by 15% in the older Ahsg<sup>−/−</sup> mice as compared with wild type and heterozygous littermates (Fig. 3, C and D). Mechanical properties of femurs were measured at 3–4 and 12–18 months of age using a three-point bending test. Maximum load normally increases with age as we observed here for all genotypes. No differences between genotypes were observed in young mice, but the older Ahsg<sup>−/−</sup> mice displayed a higher maximum load, consistent with greater BMD and femoral cortical thickness in these mice (Fig. 3E). The energy to fracture normally decreases with age, but this value increased significantly in the older Ahsg<sup>−/−</sup> mice (Fig. 3F).

**Osteogenesis and Adipogenesis Is Enhanced in Bone Marrow of Ahsg<sup>−/−</sup> Mice**—TGF-β/BMP can induce progenitor cells in bone marrow cultures to differentiate along different cell lineages depending on the cytokine concentrations (12, 25). Adipocytes in the bone marrow of Ahsg<sup>−/−</sup> mice were increased 5-fold compared with wild type and heterozygous mice (Fig. 4, A and B). Osteoblasts per trabecular surface were also increased by 60% in Ahsg<sup>−/−</sup> mice, whereas the osteoclasts per trabecular surface were not significantly different (Fig. 4, C and D). These observations suggest that progenitor cell recruitment along adipogenic and osteogenic lineages occurs at a higher frequency in Ahsg<sup>−/−</sup> mice compared with age-matched Ahsg<sup>+/+</sup> and Ahsg<sup>+/−</sup> mice. Furthermore, increased osteoblast content and cortical bone thickness indicate that net bone formation rates were enhanced in Ahsg<sup>−/−</sup> mice (Fig. 3B and 4C).

To measure directly the dynamic parameters of bone remodeling, newly forming bone was pulse-labeled with two intravenous injections of tetracycline administered 7 days apart (Fig. 4E). The mineralizing surface and mineral formation rates were increased 50 and 100% in Ahsg<sup>−/−</sup> mice compared with Ahsg<sup>+/+</sup> and Ahsg<sup>+/−</sup> mice, respectively (Fig. 4, F and G), consistent with observed differences in osteoblasts/surface (Fig. 4C). Surprisingly, mineralizing surface and mineral formation rates were significantly decreased in Ahsg<sup>−/−</sup> mice compared with control mice (Fig. 4, F and G). Correspondingly, mineralization lag time was decreased in Ahsg<sup>−/−</sup> and increased in Ahsg<sup>+/−</sup> compared with wild type mice (Fig. 4H).

The histomorphometric measurements of trabecular bone at 3 months of age also indicate an unusual relationship between the ASHG genotypes. Although Ahsg<sup>−/−</sup> and Ahsg<sup>+/−</sup> mice were similar, the heterozygous mice differed significantly for several parameters. Trabecular number and trabecular surface

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**Fig. 4.** Osteogenesis, adipogenesis, and bone remodeling is altered in Ahsg<sup>−/−</sup>, Ahsg<sup>+/−</sup>, and Ahsg<sup>+/+</sup> mice. A, toluidine blue staining of femoral sections reveals adipocytes in marrow as white areas. B, adipocytes per field, osteoblasts per bone surface, and osteoclasts per bone surface were quantified. E, dynamic properties of bone remodeling in Ahsg<sup>−/−</sup>, Ahsg<sup>+/−</sup>, and Ahsg<sup>+/+</sup> mice were examined by dual tetracycline labeling. The arrows mark the two labeled fronts observed by fluorescence microscopy. F-H, mineralizing surface/bone surface, mineral formation rate, and mineralization lag time were measured. Results are the mean ± S.E. of 12 Ahsg<sup>−/−</sup>, 8 Ahsg<sup>+/−</sup>, and 12 Ahsg<sup>+/+</sup> mice, and *, p < 0.005 versus Ahsg<sup>+/+</sup>.

Because polymorphic differences in the coding region of human AHSG correlate with differences in stature, we examined femur growth plate histology and dimensions in the mice. Balanced groups of littermates representing all three genotypes were used throughout our studies. The femur growth plates in 3-month-old Ahsg<sup>−/−</sup> mice were fragmented, and chondrocytes within the growth plate were poorly organized (Fig. 2, A and B). Twice as many discontinuities or breaks in growth plates were observed in Ahsg<sup>−/−</sup> compared with Ahsg<sup>+/+</sup> mice, and cartilage islands in the metaphysis were 6-fold more frequent in Ahsg<sup>−/−</sup> mice (Fig. 2, C and D). The cartilage islands failed to calcify as indicated by lack of von Kossa staining, which could indicate a failure of osteoclast remodeling (data not shown). Chondrocytes are normally arranged in vertical columns in the growth plate, where they undergo a spatially precise program of differentiation regulated by Indian hedgehog (Ihh), parathyroid hormone-related peptide, and BMP cytokines (34–36). Chondrocyte appeared disorganized (Fig. 2, E and F), and the number of columns per growth plate was reduced by 53% in Ahsg<sup>−/−</sup> and unchanged in Ahsg<sup>+/−</sup> compared with wild type mice (Fig. 2G). Hypertrophic chondrocytes per growth plate were reduced in Ahsg<sup>−/−</sup> mice but significantly increased in Ahsg<sup>+/−</sup> mice (Fig. 3H), whereas total chondrocyte cell counts per growth plate were not significantly different between genotypes (data not shown).
were increased and trabecular separation reduced in Ahsg⁻/⁻, whereas total trabecular bone volume was not significantly different between genotypes (Table I). Therefore, the trabecular lattice in Ahsg⁻/⁻ mice appeared to be of a finer meshwork than that observed in either wild type or ASHG-deficient mice.

Enhanced Ectopic Bone Formation in Ahsg⁻/⁻ Mice—ASHG binds to BMP-2 with a $K_d$ of $10^{-8} \text{ M} \times 10$ (10) and inhibited BMP-2-stimulated osteogenesis in rat bone marrow cell cultures (Fig. 5A). Lacking the antagonist activity, ASHG-deficient mice were expected to be more susceptible to the bone morphogenic activity of exogenous cytokine. To test the hypothesis in vivo, mice were implanted intramuscularly with pellets containing bone morphogenic cytokines, and ectopic bone formation was measured 4 weeks later. Mineral content and mineralized area at the implant site were significantly greater in Ahsg⁻/⁻ mice compared with Ahsg⁺/+ mice (Fig. 5, B and C). The mineralized area displayed the morphology of bone, with osteoblasts on the bone surface, encased osteocytes, and marrow-like compartments (Fig. 5, D and E). Thus, exogenous cytokine induced a greater area of ectopic osteogenesis in Ahsg⁻/⁻ and Ahsg⁺/+ mice, confirming an interaction between ASHG and cytokine in the regulation of osteogenesis in vivo.
ASHG is a TGF-β/BMP antagonist that localizes to mineralized bone, and here we have examined ASHG-deficient mice for bone defects that might be associated with a hyperactive cytokine environment. Chondrocyte differentiation and organization in the growth plate was impaired, and this was accompanied by slower longitudinal bone growth in Ahsg−/− mice compared with Ahsg+/+ and Ahsg+/− mice. An excess of cytokine activity in the Ahsg−/− growth plate may cause this phenotype, as exogenous TGF-β1 has been shown to prevent terminal differentiation of cells in growth plate explants into hypertrophic chondrocytes (37). The disruption of chondrocyte organization in the epiphyseal growth plate of Ahsg−/− mice may also be due in part to a defect in positioning of prechondrocytes into columnar structures. In this regard, mice lacking the BMP antagonist noggin are defective in joint formation due to an apparent failure of articular chondrocytes to position correctly (16). As cells move toward a source of agonist, the gradient strength can improve the accuracy of cell chemotaxis and achieve precise positioning of the cell at the destination. An antagonist primarily limits the effective range of an agonist, but when the two emanate from different positions, the gradient strength can improve the accuracy of cell chemotaxis to an apparent failure of articular chondrocytes to position correctly (16). As cells move toward a source of agonist, the gradient strength can improve the accuracy of cell chemotaxis and achieve precise positioning of the cell at the destination. An antagonist primarily limits the effective range of an agonist, but when the two emanate from different positions, the gradient strength can improve the accuracy of cell chemotaxis to an apparent failure of articular chondrocytes to position correctly (16).

Ahsg−/− mice were found to be more susceptible to exogenous bone morphogenic cytokines, consistent with the absence of a BMP/TGF-β antagonist in vivo. The area of ectopic osteogenesis was larger in both Ahsg−/− and Ahsg+/− mice, indicating that BMP activity extended further from the implant site when ASHG was depleted. BMP-2 can induce differentiation of mesenchymal cells into both osteoblasts and adipocytes in proportions that depend upon cytokine receptor expression and growth conditions (39). Both osteoblasts on trabecular bone and adipocytes in bone marrow were markedly increased in Ahsg−/− mice, indicating an enhanced recruitment of bone marrow mesenchymal cell precursors. This was associated with increased trabecular bone remodeling in Ahsg−/− mice, a progressive increase in cortical bone thickness, BMD, and greater maximum load to fracture in older mice. In an earlier study, Ahsg−/− mice were found to be more susceptible to spontaneous soft tissue mineralization (23) and attributed to the direct inhibition of hydroxyapatite formation by ASHG. However, ASHG blocks differentiation well before mineralization in bone marrow cultures, as indicated by a lack of osteocalcin, osteopontin, and alkaline phosphatase gene expression (12). Furthermore, addition of ASHG to the bone marrow cultures after differentiation, but prior to mineralization, did not inhibit the latter. The ectopic bone formed in response to bone morphogenic cytokines in Ahsg−/− mice displayed the typical bone morphology, consistent with an osteogenic process rather than accumulation of soft tissue calcification. However, it is possible that ASHG regulates both cytokine-dependent osteogenesis and the final stage of mineralization.

The bone phenotype in Ahsg−/− mice has features that are comparable with genetic mutations affecting TGF-β cytokines. Missense mutations of TGF-β1 latency-associated peptide cause enhanced activation of TGF-β1 in Camurati-Engelmann disease, an autosomal dominant disorder characterized by hyperostosis and sclerosis of the diaphysis of the long bones (40). BMP-3 has recently been shown to antagonize the osteogenic BMPs, and BMP3−/− mice show a 2-fold increase in trabecular bone (41). TGF-β1-deficient mice display reduced bone mass and elasticity, as well as growth plate defects (42). Furthermore, a polymorphism in the coding region of the human TGF-β1 gene has been correlated with decreased serum levels of TGF-β1 and susceptibility to osteoporosis in postmenopausal Japanese women (43). Interestingly, transgenic mice expressing TGF-β2 in osteoblasts show bone loss (44), and mice expressing dominant negative TpRII in osteoblasts display an increase in trabecular bone (45). However, the phenotypes are complex, as TGF-β2 transgenic mice also show increased osteoblast and osteocyte differentiation, which may be in part dependent on TGF-β-mediated increases in osteoclastic activity (46).

TGF-β1 is a negative regulator of lymphocyte proliferation and inflammation (47), and therefore loss of systemic antagonists might be expected to result in immune suppression. Mice lacking α2-macroglobulin, another serum TGF-β-binding glycoprotein, display a hypo-inflammatory phenotype and resistance to endotoxin challenge (48). Ahsg−/− mice are also more resistant to endotoxin than Ahsg+/+ mice. T cells from Ahsg−/− mice are less responsive to stimulation by anti-CD3 and anti-CD28 antibodies, and in addition, skin inflammation induced by topical application of arachidonic acid was reduced and returned to normal more quickly in Ahsg−/− compared with Ahsg+/+ mice. Interestingly, Ahsg gene expression is down-regulated by interleukin-1 and interleukin-6 in hepatic cells (49), and ASHG is known to be a reverse acute-phase reactant (7). It is possible that down-regulation of ASHG enhances TGF-β-mediated immune suppression. Elevated interleukin-6 is also associated with osteoporosis in inflammatory bowel disease due to increased osteoclastic activity (50); an associated reduction in bone ASHG could also affect osteogenesis.

For some femur characteristics, ASHG heterozygous mice were not intermediate between Ahsg−/− and Ahsg+/+ mice, notably maturation of growth plate chondrocytes, static parameters of trabecular bone, and dynamic measures of bone remodeling. One interpretation is that ASHG can exert both a positive and a negative effect that influence these phenotypes. For example, a cytokine buffering or carrier activity might be reflected in the observed phenotype of Ahsg+/− mice, whereas loss of the cytokine antagonist activity dominates the observed phenotype in the Ahsg−/− bone. In spatial terms, the concentration of ASHG and cytokines in bone and their release with remodeling could establish cytokine gradients in the bone marrow.

The Ahsg−/− mice would lack the ASHG-dependent cytokine gradient, resulting in an enlarged zone of stromal cell recruitment, which is consistent with the observed increase in osteoblast and adipocyte content throughout the marrow of Ahsg−/− mice (Fig. 4). A 2–3-fold reduction in serum ASHG and near wild type levels of ASHG in mineralized bone of Ahsg+/− mice should result in a steeper ASHG gradient and, consequently, a more narrow region where cytokine levels are optimal for osteogenesis. Consistent with this model, trabecular bone in Ahsg−/− mice displayed a significantly finer meshwork as well as reduced remodeling rates compared with that in either Ahsg+/+ or Ahsg−/− mice (Table I). However, the interactions are likely to be more complex, as ASHG binds to multiple TGF-β/BMP cytokines with different affinities and therefore has the potential to change cytokine activities in a complex manner that could readily give rise to a distinct Ahsg+/− phenotype. In any event, detection of an Ahsg+/− phenotype suggests that a relatively modest change in ASHG levels can affect bone remodeling rates and trabecular architecture.

Bone TGF-β levels decline with age (28, 29), which may lead to an imbalance with antagonists and loss of cytokine-dependant stromal cell recruitment. However, TGF-β1 cytokine expression in tissue fibroblasts increases with age (51), and an imbalance relative to antagonists may promote ectopic osteo-

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genesis, immune suppression, and fibrosis in the elderly. TGF-β1 contributes to pathologies associated with atherosclerosis, kidney disease, chronic obstructive pulmonary disease in asthma patients, diabetic nephropathy, vitreoretinopathy, scar formation in wound healing (52, 53), and also tumor progression (54). We have observed that intestinal tumor progression was enhanced in Ahsg−/− compared with Ahsg+/+ mice with an Apc mutation (Min+/−). Thus, TGF-β antagonists or blockers of cytokine signaling may have therapeutic applications in advanced cancers and fibrosis. Lending further support to this notion, genetic deletion of Smad3, which mediates TGF-β cytokine signaling may have therapeutic applications in ad-

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