Microfibril-associated Glycoprotein-1, an Extracellular Matrix Regulator of Bone Remodeling

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MAGP1 is an extracellular matrix protein that, in vertebrates, is a ubiquitous component of fibrillar-rich microfibrils. We previously reported that aged MAGP1-deficient mice (MAGP1Δ) develop lesions that are the consequence of spontaneous bone fracture. We now present a more defined bone phenotype found in MAGP1Δ mice. A longitudinal DEXA study demonstrated age-associated osteopenia in MAGP1Δ animals and μCT confirmed reduced bone mineral density in the trabecular and cortical bone. Further, MAGP1Δ mice have significantly less trabecular bone, the trabecular microarchitecture is more fragmented, and the diaphyseal cross-sectional area is significantly reduced. The remodeling defect seen in MAGP1Δ mice is likely not due to an osteoblast defect, because MAGP1Δ bone marrow stromal cells undergo osteoblastogenesis and form mineralized nodules. In vivo, MAGP1Δ mice exhibit normal osteoblast number, mineralized bone surface, and bone formation rate. Instead, our findings suggest increased bone resorption is responsible for the osteopenia. The number of osteoclasts derived from MAGP1Δ bone marrow macrophage cells is increased relative to the wild type, and osteoclast differentiation markers are expressed at earlier time points in MAGP1Δ cells. In vivo, MAGP1Δ mice have more osteoclasts lining the bone surface. RANKL (receptor activator of NF-κB ligand) expression is significantly higher in MAGP1Δ bone, and likely contributes to enhanced osteoclastogenesis. However, bone marrow macrophage cells from MAGP1Δ mice show a higher propensity than do wild-type cells to differentiate to osteoclasts in response to RANKL, suggesting that they are also primed to respond to osteoclast-promoting signals. Together, our findings suggest that MAGP1 is a regulator of bone remodeling, and its absence results in osteopenia associated with an increase in osteoclast number.

Originally thought to serve a purely structural role, the extracellular matrix (ECM) is now known to be an important regulator of tissue development and homeostasis. Microfibrils are an abundant component of the ECM and can be found alone as microfibril bundles or associated with elastin in elastic fibers. Three fibrillins (FBN1, -2, and -3) provide the major structural components of these 10 nm diameter fibrils (1–3), although numerous microfibril-associated proteins interact with fibrillin and contribute to microfibril function. Fibrillin expression is widespread throughout development and is a product of most mesenchymal/interstitial cells (4). In the developing skeletal system, fibrillin expression has been documented in limb bud development, as well as in the adult bone (1, 5). Within the bone, fibrillin microfibrils can be found in the periosseous matrix, surrounding osteocytes, chondrocytes, and osteons, on the endochondral surface, and within the trabecular matrix (7).

Microfibrils have clinical significance as mutations in the fibrillin genes give rise to a number of heritable connective tissue disorders. Mutations in the gene for fibrillin-1, for example, are linked to Marfan syndrome, dominant ectopia lentis, and the autosomal-dominant form of Weill–Marchesani syndrome (8–11). In addition to eye defects, these diseases can include abnormalities of the axial and appendicular skeleton, craniofacial and cardiovascular defects, and altered muscularity and adiposity (reviewed in Ref. 12). Mutations in the gene for fibrillin-2 have been genetically linked to congenital contractual arachnodactyly, a rare disorder that shares some of the skeletal manifestations of Marfan syndrome (9, 13).

Fibrillin-1 mutation or deficiency in mice results in a skeletal phenotype similar to that seen in humans with Marfan syndrome (14, 15). Disruption of fibrillin-1 expression leads to rib and long bone overgrowth and kyphosis. Significant loss in cortical and trabecular bone volume, reduced femoral bone mineral density, altered trabecular microarchitecture, and a defect in osteoblastogenesis were also reported in fibrillin-1-mutant tight skin (Tsk) mice (15). Fibrillin-2 deficiency in mice mimics the human congenital contractual arachnodactyly phenotype, and this mouse model implicates fibrillin-2 as a regulator of limb patterning (16). Providing a potential mechanism for dis-
ease pathogenesis, recent studies with these animal models directly connect fibrillin mutations with dysfunction of TGFβ and BMP (16–19). The large latent complex of TGFβ localizes to microfibrils in the ECM of tissues and cells (20), and latent TGF-β binding protein-1 was shown to interact with a sequence near the amino terminus of fibrillin-1 (21, 22). Several BMPs through their pro-domain also interact with fibrillin-1 at the same region (23). Antagonism of TGFβ BMPs through their pro-domain also interact with fibrillin-1 at sequence near the amino terminus of fibrillin-1 (21, 22). Several TGF-

ated with TGF-II, Shprintzen-Goldberg syndrome, and other diseases associ-

tion of these binding sites provides insight into MAGP1 func-

second site in the middle of the molecule (36–40). The loca-

with fibrillin at a sequence near fibrillin’s N terminus and at a

other EGF-containing molecules (35).

Protein binding studies demonstrate that MAGP1 interacts

fibrillin at a sequence near fibrillin’s N terminus and at a

other EGF-containing molecules (35).

Like other ECM proteins, MAGP1 is organized into multiple

functional domains. The N-terminal half of the protein con-

contains a cluster of acidic and sulfated tyrosine residues that
define a binding domain for cationic proteins, such as growth

factors like TGFβ and BMPs. In contrast to fibrillin-1, which

bonds latent forms of the TGFβ growth factor family, MAGP1

bonds active TGFβ and BMPs (34). The back half of MAGP1 is

rich in cysteines and contains the protein’s matrix binding

domain, which facilitates interactions with fibrillin, notch, and

other EGF-containing molecules (35).

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**EXPERIMENTAL PROCEDURES**

**Nomenclature**—The nomenclature for the MAGPs and their
genes is somewhat confusing. The gene name for MAGP1 is

*Mfap2*, whereas the gene name for MAGP2 is *Mfap5*. To avoid

confusing MAGP1 and MAGP2 when referring to knock-out

mice, we will refer to the MAGP1 knock-out genotype

(*Mfap2<sup>−/−</sup>*) as MAGP1Δ.

**Statistical Analysis**—A paired *t* test was used to determine
the statistical significance between genotypes. Values were con-
sidered significantly different when *p* values were <0.05.

**Animals**—Generation and genotyping of the MAGP1Δ col-
ony has been described (34). Male mice were utilized for all
studies and ranged in age from 4 weeks to 6 months as indi-
cated. All mice were in the Black Swiss background (BkSw, Tac-
onic, Hudson, NY) and were housed in a pathogen-free animal
facility, fed standard chow *ad libitum*, and treated following
animal protocols approved by the Washington University Ani-
mal Studies Committee.

**BMSC Isolation and Culture**—Femurs and tibias were
removed from 3-month-old WT and MAGP1Δ mice and

of clean soft tissue, and the distal end of the bone was
removed. Marrow cells were collected by brief centrifugation of
the bone followed by incubation in red blood cell lysis buffer for
10 min at room temperature. The lysis buffer was removed, and
the remaining cells were passed through a 70-μm cell filter,
than cultured in α-MEM containing antibiotics and 10% fetal
bovine serum (OB growth media). For osteogenic differentia-
tion, cells were cultured in osteogenic media (OB growth media
supplemented with 50 μg/ml ascorbic acid and 10 mM β-glyc-
erolphosphate). The medium was replaced biweekly. Cells were
lysed for RNA analysis or fixed for alkaline phosphatase activity
at day 7. Cells for mineralization assays were fixed and stained
using Alizarin Red on day 28. Alkaline phosphatase staining
was performed using a Sigma-Aldrich kit (85L1) according to
the manufacturer’s instructions.

**BMM Isolation and Culture**—Femurs and tibias were
removed from 4- to 6-week-old WT and MAGP1Δ mice,
cleaned of soft tissue, and flushed with α-MEM to recover the
bone marrow. Marrow cells were cultured on plastic dishes in
α-MEM containing penicillin/streptomycin, 10% inactivated
fetal bovine serum, and 1:10 CMG (conditioned media contain-
ing macrophage-colony stimulating factor (43)). After 4 days in
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culture, the adherent bone marrow macrophage cells (BMMs) were trypsinized and re-plated into tissue culture-treated plastic dishes containing OC differentiation media (α-MEM, penicillin/streptomycin, 10% inactivated fetal bovine serum, 1:50 CMG, and 50 ng/ml recombinant glutathione S-transferase-RANKL). The cells were grown for an additional 4–5 days under these conditions then stained for TRAP (Sigma-Aldrich) or lysed for protein analysis. TRAP staining was performed as previously described (44).

Calvaria Osteoblast Isolation and Culture—Whole calvaria were extracted from 4-day-old pups and cleaned of soft tissue. Calvaria from 4 pups per genotype were pooled. Osteoblasts were liberated by serial collagenase treatment. BMMs from 4-week-old mice were harvested on the same day (described above). Osteoblasts and BMMs were cultured for 4 days prior to being lifted and cultured together for 5–6 days in α-MEM media containing penicillin/streptomycin, 10% fetal bovine serum, 10 nM VitD3.

RNA Extraction and Quantitative RT-PCR—RNA from cultured cells was isolated and purified using a Qiagen (Valencia, CA) RNeasy RNA purification kit; RLT lysis buffer was supplemented with β-mercaptoethanol (1%). Purified RNA was treated with DNase I (Invitrogen) prior to reverse transcription (RT). RT was performed using SuperScript III (Invitrogen) and quantitative PCR (qPCR) was performed using Power SYBR green master mix (Applied Biosystems, Foster City, CA) and gene-specific primers. The qPCR reaction was run on ABI Prism 7000 (Applied Biosystem). Transcript levels (relative to cyclophilin), normalized to cyclophilin, were determined by the equation, 1/(2−ΔΔCT)*10,000. qPCR was performed on whole bone tissue as described above, with the following modifications. Marrow-flushed trabeculae from 6-month-old mice, or calvaria from 6-week-old mice, were cleaned of soft tissue, and snap frozen. Bones were pulverized using a Braun Mikrodisembrator. Pulverized tissue was collected in TRIzol, and RNA was extracted with chloroform and further purified using the Qiagen RNeasy kit described above.

Whole Body Bone Mass (DEXA)—WT and MAGP1Δ mice were injected with 7.5 mg/kg calcein (Sigma-Aldrich). Seven days later mice were injected with 30 mg/kg alizarin 3-methyl iminodiacetic acid, and trabecular bone measurements were taken after an additional 2 days for histologic analysis. Dynamic assessment of trabecular bone formation was determined on non-decalcified MMA sections based on single and dual calcein-alizarin labeling using BioQuant software (BioQuant, Nashville, TN). This software provided measures of bone surface, percent single- and double-labeled bone surface (BS), mineralizing surface, mineral apposition rate, and bone formation rate. Trabecular bone measurements were taken from a region encompassing a 800-μm-long field, across the width of the bone (located 50–550 μm below the growth plate). For each animal, two serial sections were analyzed, and the measurements were averaged (n = 12 for WT and 8 for MAGP1Δ). Pictures were obtained using an Olympus BX51 fluorescent microscope fitted with a DP70 camera (Olympus, Center Valley, PA).

Quantitative Histomorphometry—TRAP and hematoxylin and eosin-stained tibial sections were used to visualize osteoclasts and osteoblasts, respectively, using the Olympus BX51 light microscope and camera described above (Olympus). Trabecular osteoblasts and osteoclasts were measured in a 500-μm field across the bone in an area located 50 μm distal to the growth plate using BioQuant software. Measurements included osteoclast number/BS and osteoblast number/BS.

Western Blotting—Cultured BMM cells were lysed in buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 mM NaF × protease
inhibitor (Roche Applied Science). Lysates were cleared of cellular debris by centrifugation, and protein concentration was determined. Lysate was run on 10% SDS-PAGE gels then transblotted to nitrocellulose membranes. Membranes were blocked in 1% casein then incubated overnight with the indicated primary antibodies. Proteins were visualized through use of fluorescence-labeled secondary antibodies and the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

Bone Mechanical Testing (3-Point Bend)—Femurs were thawed in phosphate-buffered saline for 1 h prior to use, and testing was carried out at room temperature using a servohydraulic testing machine (8841 Dynamite, Instron, Norwood, MA). Femurs were positioned on two supports 7 mm apart, and the central loading point was mid-diaphysis. Displacement was applied transverse to the long axis of the bone at a rate of 0.03 mm/s until failure. Force-displacement data were recorded at 60 Hz and analyzed to determine measures of stiffness (rigidity) and whole bone strength (ultimate moment) (45).

Bone-resorptive Pit Staining—BMMs were cultured on bovine bone slices in the presence of macrophage-colony stimulating factor and RANKL for 6 days. Mechanical agitation was used to remove the cells from the bone slices. The bone slices were then incubated with peroxidase-conjugated wheat germ agglutinin (Sigma) then 3,3-diaminobenzidine (Sigma). Resorption lacunae size was determined from the average of five fields from four chips per mouse, and three mice per genotype.

RESULTS

MAGP1-deficient Animals Have Reduced Bone Strength and Become Osteopenic with Age—We (Fig. 1A) and others (46) have demonstrated MAGP1 to be abundantly expressed in bone. MAGP1-deficient mice, however, appear to have normal skeletal development, and tibia length measurements demonstrate only a slight overgrowth in the MAGP1-deficient animals (Fig. 1B). We previously reported that a percentage of aged MAGP1-deficient mice develop lesions on their lower limbs consistent with bone fracture (34). Using the 3-point bend test, we were able to confirm that femurs from MAGP1−/− mice have reduced whole bone strength and rigidity (Fig. 1C).

To better understand why MAGP1−/− bones are more susceptible to failure, we performed a longitudinal DEXA scan study following mice from 4 to 24 weeks old. The findings show that MAGP1−/− mice were unable to achieve the BMD or bone mineral content levels seen in WT mice (Fig. 2, A–C). To determine BMD using μCT, 30–16 μm slices were contoured to determine trabecular BMD (Tb.BMD, E), and 10–16 μm slices were contoured to determine cortical BMD (Ct.BMD, F). The μCT data are presented as mean ± S.D. (*, p < 0.05; **, p < 0.01; +/+ , n = 10; −/− , n = 9).
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was reduced 40% in MAGP1Δ animals compared with only a 5% reduction in cortical BMD (Fig. 2, E and F).

MAGP1Δ Mice Have Significantly Less Trabecular Bone and Disrupted Microarchitecture—Histological and μCT analyses confirmed that the trabecular bone was significantly affected by MAGP1 deficiency. Histology on longitudinal tibia sections revealed a significant reduction in trabecular bone (Fig. 3A). Magnification showed that MAGP1Δ mice have 56% less trabecular bone surface compared with WT (Fig. 3B). Based on μCT analysis of the tibia epiphyses, trabecular BMD was reduced 40% in MAGP1Δ animals (Fig. 2E). Consistent with the reduction in volumetric BMD, three-dimensional images clearly demonstrate significantly less trabecular bone mass in the MAGP1Δ mice (Fig. 3C). Quantitatively, there is a 58% reduction in the ratio of bone volume to tissue volume (BV/TV, Fig. 3D).

Further, measures of trabecular microarchitecture were altered in the MAGP1Δ mice. The trabecular connectivity density (Conn.Dens.) was reduced 76% compared with WT (Fig. 4A). The structure model index was increased from 1.6 to 2.6 in the bones of MAGP1Δ animals, indicating an increase in the ratio of rod-like to plate-like trabeculae (Fig. 4B). Trabecular separation (Tb.Sp., Fig. 4C) was significantly increased in the knockouts. There was also a slight reduction in trabecular thickness (Tb.Th., Fig. 4D), but no difference in the trabecular number (Tb.N., Fig. 4E). These data indicate that MAGP1-deficient mice have diminished trabecular bone mass and that the trabecular network is fragmented and with thinner trabeculae relative to WT. These findings demonstrate that MAGP1 is a regulator of bone remodeling.

MAGP1Δ Mice Have Altered Cortical Bone Morphology and Density—μCT of the tibial diaphysis revealed altered cortical morphology (Fig. 5A). Cortical mineral density (BMD) was reduced slightly (−5%) but significantly (Fig. 4F). Analysis of diaphyseal cross-sections revealed that cortical bone area was reduced −9% (Ct.Ar., Fig. 5B) and cortical bone width was reduced −2% (Ct.Wi., Fig. 5C). These modest changes in the amount of cortical bone were accompanied by a significant reduction of 33% in the medullary area of MAGP1Δ bones (Ma.Ar., Fig. 5D). Accordingly, tissue area was reduced 16% (T.Ar., Fig. 5E). The large reduction in cross-sectional bone size is consistent with reduced whole bone strength reported in the bending studies (Fig. 1C). Together, the morphologic analyses demonstrate that MAGP1 is involved in skeletal remodeling and maintenance.

Osteoblasts Function Normally in the Absence of MAGP1—To delineate the mechanism underlying reduced bone volume and BMD, bone marrow stromal cells (BMSCs) were isolated from WT and MAGP1-deficient mice to determine their propensity to differentiate into osteoblasts. Following 7 days of incubation in osteogenic medium, differentiated MAGP1Δ cells expressed osteoblast gene markers at levels similar to (RUNX2, OC, and OSX), or sometimes greater (bone sialoprotein and Col1a1) than, that of the WT cells (Fig. 6A). MAGP1Δ BMSCs also stained positive for alkaline phosphatase activity at levels comparable to WT BMSCs (Fig. 6B). Further, following 28 days in osteogenic media there was no difference in the mineralization potential of MAGP1Δ cells (Fig. 6C).

Analysis of histologic sections found no significant difference in the number of osteoblasts lining the trabecular bone surface (Figs. 6D). Dynamic indices of bone formation were measured in WT and MAGP1Δ mice using dual calcine-alizarin labeling. There was no difference in the ratio of mineralizing surface to bone surface (MS/BS, Fig. 6E). There was only a slight, but not significant, reduction in both mineral apposition rate and the bone formation rate to bone surface ratio (data not shown). These findings imply that the osteopenia of MAGP1-deficient mice does not result from a defect in either osteoblastogenesis or osteoblast function.

MAGP1 Deficiency Results in Increased Osteoclast Number, but Normal Osteoclast Function—Increased bone loss in the context of normal osteoblast number and function suggests
that increased bone resorption might be the cause of the osteopenic phenotype. Indeed, BMMs isolated from MAGP1Δ mice produced a significantly higher number of osteoclasts after 4-day exposure to RANKL (50 ng/ml) relative to WT BMMs (Fig. 7A). Enhanced osteoclastogenesis was also evaluated by monitoring osteoclast differentiation markers over time. β3-integrin and c-Src, markers of commitment to the osteoclast lineage, were visualized via Western blot, and both proteins were found to be expressed at higher levels and at earlier time points in the MAGP1-deficient cells (Fig. 7B).

To determine if the resorptive capacity of individual mutant polykaryons is enhanced, we generated WT and MAGP1Δ osteoclasts on bone and measured the area of pits they excavated. In contrast to osteoclast number, the average pit area per cell is indistinguishable between genotypes (Fig. 7C).

To determine whether an increase in osteoclast number is also present in MAGP1-deficient mice in vivo, quantitative histomorphometry was performed on TRAP-stained tibia sections. The results confirmed that MAGP1Δ mice have 57% more osteoclasts lining the trabecular surface when compared with WT mice (Fig. 7D). These findings suggest that MAGP1 functions to inhibit osteoclast differentiation such that, when absent, there is an increase in the ratio of osteoclasts to osteoblasts.

**MAGP1Δ BMMs Are Primed for Osteoclastogenesis—** MAGP1 is abundantly expressed in the bone. Utilizing RT-qPCR it was determined that committed osteoblasts, not cells of the osteoclast lineage, are the major producers of MAGP1 (Figs. 8, A and B). Thus, it was important to determine whether culturing WT osteoblasts with MAGP1Δ BMM could reverse the enhanced osteoclastogenesis associated with MAGP1Δ BMM (Fig. 7A). Calvaria osteoblasts from 4-day-old WT and MAGP1Δ pups were cultured with either WT or MAGP1Δ BMMs. After 5–6 days of co-culture, osteoblasts were removed, and the remaining osteoclasts were visualized by TRAP stain. As seen in Fig. 9A (day 5), co-culturing OB and BMM from MAGP1Δ mice results in significantly more osteoclasts relative to WT cells. Interestingly, culturing MAGP1Δ BMM with WT OB only partially reversed this phenomena, and culturing WT BMM with MAGP1Δ OB had little effect on osteoclastogenesis. However, by day 6 of coculture (Fig. 9B) WT OB with MAGP1Δ BMM are indistinguishable from KO-KO cultures, and MAGP1Δ OB plus WT BMM are then exhibiting greater osteoclast numbers than WT-WT cultures. From this we conclude that an in vivo priming of the BMMs to respond to RANKL is occurring and that this effect predominates over the MAGP1Δ osteoblast-mediated enhanced osteoclastogenesis.

**Elevated RANKL Expression in MAGP1Δ Osteoblasts—** Osteoblast-derived RANKL binding to RANK is essential for directing macrophages down the osteoclast lineage and thus bone resorption. OPG,
a RANKL decoy receptor, blocks RANKL–RANK binding and thus bone turnover (47). The RANKL/OPG ratio is considered an important predictor of bone mass (48). We therefore evaluated whether MAGP1 deficiency augmented the RANKL/OPG system. RANKL and OPG transcript levels were determined from cultured calvaria osteoblasts (Fig. 9C) and whole calvaria tissue (Fig. 9D). We found cultured calvaria osteoblasts from MAGP1Δ mice expressed 14-fold more RANKL relative to WT cells. We were also able to detect a 3-fold increase in RANKL in MAGP1Δ whole calvaria. In both experiments, there was no difference in OPG expression between genotypes.

**DISCUSSION**

One of the most intriguing phenotypes in aged MAGP1Δ mice is the appearance of lesions on their lower limbs that are indicative of spontaneous bone fracture (34). Femoral mechanical testing reported in this current study confirmed that bones from MAGP1Δ mice are more susceptible to failure relative to WT. μCT analysis revealed MAGP1Δ cortical bone to have reduced mineralization and cross-sectional size, which is consistent with their reduced whole bone stiffness and strength determined by bending test.

Trabecular bone remodels at a faster rate than cortical bone. Consequently, if MAGP1 deficiency causes dysregulation of bone turnover, it will be most apparent in the trabeculae. Interruptions in the trabecular network result in a reduced surface for osteoblasts to replace resorbed bone, and disturbing the trabecular network can result in reduced load bearing capacity thereby increasing susceptibility to fracture. Individuals with osteoporosis, for example, are most likely to fracture where
trabecular bone dominates (49). We found MAGP1Δ mice to have abnormal trabecular microarchitecture consistent with osteopenia. Trabecular BMD and bone volume in these animals are significantly reduced. Further, the trabeculae have fewer interconnections and have a more rod-like morphology as opposed to a plate-like morphology. These changes in both the trabecular and cortical bone are consistent with the bone fragility seen previously in MAGP1Δ mice (34).

Osteoblasts in MAGP1Δ Mice Are Normal in Number and Function—The balance of bone formation by osteoblasts and bone resorption by osteoclasts is key to maintaining bone integrity. When BMSC were induced to differentiate in vivo, alkaline phosphatase activity, mineralized nodule formation, and expression of osteoblast differentiation markers were essentially equivalent in cells from MAGP1Δ and WT BMSC animals. When the number of osteoblasts was normalized to bone surface size there was no significant difference between the two genotypes. Similarly, mineralized surface, mineral apposition rate, and bone formation rates were unaffected by MAGP1 deficiency. Together, these data support normal osteoblast differentiation, number, and bone forming ability in the absence of MAGP1. MAGP1 not playing a role in osteoblast differentiation is consistent with it being produced by mature osteoblasts after differentiation has occurred (Fig. 8A).

Elevated Number of Normal Osteoclasts in MAGP1Δ Bone—The presence of normal osteoclasts in MAGP1Δ-deficient bone suggests that enhanced bone loss is due to a shift in the balance of bone remodeling toward osteoclasts and bone resorption. Indeed, our studies documented significantly more osteoclasts lining the trabecular bone surface in MAGP1-deficient animals, which correlated with a significant loss of trabecular bone volume and thickness, and greater trabecular bone separation. An explanation for the increased osteoclast number was suggested by in vitro experiments wherein more osteoclasts were derived from MAGP1Δ BMMS than WT BMMS in response to stimulation with RANKL. Further, MAGP1Δ BMMS expressed osteoclast differentiation markers earlier in response to RANKL stimulation. The response seen in these BMMS cultures suggests that MAGP1Δ BMMS are sensitized to RANKL and, hence, are primed for osteoclastogenesis. Pit forming assays on whole bone showed that the osteoclasts from MAGP1-null animals were functionally normal. Because BMMS express insignificant amounts of MAGP1 relative to osteoclasts, the MAGP1 effects on osteoclastogenesis are likely derived from microfibrils produced by mature osteoblasts (Fig. 8, A and B).
null mouse. Indeed, mice carrying the autosomal dominant tight skin mutation (Ts[k/]) have a genomic duplication within the fibrillin-1 gene that results in an in-frame duplication of exons 17–40, which includes a region encompassing one of fibrillin’s MAGP1 binding sites (42). The mutant protein is produced in normal amounts and when combined with fibrillin from the WT allele forms a microfibril of altered structure and function. Although there are phenotypic differences between Ts[k/] and MAGP1Δ mice, the skeletal phenotype is strikingly similar in the two animals (15), suggesting that alterations in MAGP1 binding might contribute to the phenotype.

Summary—we have identified MAGP1 to be an important regulator of bone remodeling. In the absence of MAGP1, mice develop osteopenia, have significantly less trabecular bone and altered cortical bone modeling relative to age-matched controls. Our data indicate that the reduced mineralization and bone volume associated with MAGP1 deficiency is the consequence of an uncoupling in bone formation and resorption. More specifically, osteoblast-mediated bone formation remains normal, whereas an increased osteoclast number can lead to enhanced bone resorption. These findings support a role for microfibrils (fibrillin plus MAGP1) in bone remodeling and homeostasis.

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