Full Length Article

Induction of Lrp5 HBM-causing mutations in Cathepsin-K expressing cells alters bone metabolism

Kyung Shin Kang, Jung Min Hong, Daniel J. Horan, Kyung-Eun Lim, Whitney A. Bullock, Angela Bruzzaniti, Steven Hanne, Matthew L. Warman, Alexander G. Robling

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ABSTRACT

High-bone-mass (HBM)-causing missense mutations in the low density lipoprotein receptor-related protein-5 (Lrp5) are associated with increased osteoanabolic action and protection from disuse- and ovariectomy-induced osteopenia. These mutations (e.g., A214V and G171V) confer resistance to endogenous secreted Lrp5/6 inhibitors, such as sclerostin (SOST) and Dickkopf homolog-1 (DKK1). Cells in the osteoblast lineage are responsive to canonical Wnt stimulation, but recent work has indicated that osteoclasts exhibit both indirect and direct responsiveness to canonical Wnt. Whether Lrp5-HBM receptors, expressed in osteoclasts, might alter osteoclast differentiation, activity, and consequent net bone balance in the skeleton, is not known. To address this, we bred mice harboring heterozygous Lrp5 HBM-causing conditional knock-in alleles to Ctsk-Cre transgenic mice and studied the phenotype using DXA, μCT, histomorphometry, serum assays, and primary cell culture. Mice with HBM alleles induced in Ctsk-expressing cells (TG) exhibited higher bone mass and architectural properties compared to non-transgenic (NTG) counterparts. In vivo and in vitro measurements of osteoclast activity, population density, and differentiation yielded significant reductions in osteoclast-related parameters in female but not male TG mice. Droplet digital PCR performed on osteocyte enriched cortical bone tubes from TG and NTG mice revealed that ~8–17% of the osteocyte population (depending on sex) underwent recombination of the conditional Lrp5 allele in the presence of Ctsk-Cre. Further, bone formation parameters in the midshaft femur cortex show a small but significant increase in anabolic action on the endocortical but not periosteal surface. These findings suggest that Wnt/Lrp5 signaling in osteoclasts affects osteoclastogenesis and activity in female mice, but also that some of the changes in bone mass in TG mice might be due to Cre expression in the osteocyte population.

1. Introduction

Most FDA-approved therapies for osteoporosis target the bone-resorbing activities of osteoclasts [1]. More recently, anabolic treatments have been considered a promising approach to improve bone properties. Approved agents that stimulate anabolic action are focused around the PTH/PTHrP axis, but discoveries regarding anabolic potential of the Wnt signaling pathway have provided other avenues to achieve anabolism in bone [2,3]. Several plasma membrane receptors are involved in the Wnt signaling pathway, including the low density lipoprotein receptor-related protein-5 (LRP5). Numerous reports indicate that LRP5 is a key protein involved in the regulation of bone mass [4]. The importance of LRP5 in skeletal regulation has been addressed using genetically engineered mouse models, all of which consistently show significant changes in bone mass and strength with gain- and loss-of-function mutations in the gene [5–9].

Much of the high bone mass (HBM) phenotype described in mice with LRP5 HBM-causing mutations (e.g. Lrp5-HBM transgene or knock-in point mutations) has been ascribed to altered anabolic signaling in the osteoblast/osteocyte. Mice with HBM-causing missense mutations...
in Lrp5 provide robust gain-of-function models because LRPS HBM receptors respond normally to Wnt ligands but exhibit resistance to endogenous inhibitors such as sclerostin (SOST) and Dickkopf homolog-1 (DKK1) [10–13]. Additionally, these mutations enhance responsiveness to mechanical loading and protect the skeleton from the bone-wasting effects of mechanical disuse [14,15].

While most of the published reports on Wnt action in bone have focused on the anabolic effects in osteoblasts, or on the anabolism-relaying effects in osteocytes, much less is known about Wnt signaling in osteoclasts. Osteoclasts are modulated by Wnt proteins, secreted by neighboring osteoblasts and osteocytes [16]. Beyond indirect effects on the osteoclast (e.g., Wnt-modulated RANKL/OPG production by osteocytes/osteoblasts), more recent data suggest that Wnt stimulation can have direct effects on osteoclasts [17]. The most thoroughly characterized mechanism for direct osteoclast stimulation by Wnt is the Wnt5a/Ror2 pathway, which activates one of the non-canonical arms of intracellular Wnt signaling [18]. However, osteoclasts also express the molecular machinery to transduce canonical Wnt signaling (e.g., LRP5/6, FZDs, β-catenin, GSK3β) [19], and deletion of LRP5/6 in early osteoclasts results in increased resorption and low bone mass, whereas treatment of osteoclast cultures with the canonical ligand Wnt3a induces β-catenin nuclear translocation and suppresses osteoclast differentiation [17]. In light of the accumulating evidence that canonical Wnt signaling might have a significant role in the osteoclast, we hypothesized that osteoclast-selective (i.e., Ctsk-Cre-mediated) expression of LRP5 receptors harboring the HBM-causing G171V or A214V mutations would exhibit increased bone mass via Wnt-mediated inhibition of osteoclasts.

In this communication, we sought to investigate whether expression of LRP5 gain-of-function mutations (G171V, A214V) in osteoclasts would alter bone homeostasis in vivo and osteoclastogenesis in vitro. Mice harboring heterozygous Lrp5 HBM-causing conditional knock-in alleles were bred with Ctsk-Cre transgenic mice. We evaluated in vivo actions of both Lrp5 mutant alleles individually, expressed in osteoclasts, on bone mass and degradation. We also explored the in vitro effects of the mutations on osteoclast differentiation and activity, using bone marrow hematopoietic stem cells isolated from the mutant mice. Here, we report that Lrp5 HBM-causing knock-in alleles in Ctsk-expressing cells significantly increase overall bone mass and reduce resorption in female mice, but have more mild effects in male mice.

2. Materials and methods

2.1. Experimental mice

Mice with Lrp5 conditional knockin alleles for HBM-causing
mutations A214V and G171V have been described previously [5].

These alleles, Lrp5<sup>AN</sup> and Lrp5<sup>GN</sup>, respectively) are activated by Cre-recombination. Male mice with conditional Lrp5 alleles were crossed to hemizygous female Ctsk-Cre transgenic (TG) mice, which is expressed in osteoclasts [20]. Offspring with the following genotypes were studied: TG;+/A (mice with the Lrp5<sup>AN</sup> allele and the Ctsk-Cre transgene), NTG; +/A (mice with the Lrp5<sup>AN</sup> allele but not the Ctsk-Cre transgene), TG; +/G (mice with Lrp5<sup>GN</sup> allele and the Ctsk-Cre transgene), and NTG; +/G (mice with the Lrp5<sup>GN</sup> allele but not the Ctsk-Cre transgene). The genetic background of all mice was a mixture of 129S1/SvIMJ and C57Bl/6J. Offspring were same sex–housed in cages of 3 to 5 (independent of Ctsk-Cre genotype) and given standard mouse chow [Harlan Teklad 2018SX; 1% Ca; 0.65% P; vitamin D3 (2.1 IU/g)] and water ad libitum. The Lrp5 alleles and the Ctsk-Cre transgene were genotyped using standard PCR on genomic DNA from ear notches. All animal procedures were performed in accordance with relevant federal
guidelines and conformed to the Guide for the Care and Use of Laboratory Animals (8th Edition). The animal facility at Indiana University is an AAALAC-accredited facility.

2.2. Dual-energy X-ray absorptiometry (DEXA)

Whole-body DEXA scans were collected on isoflurane-anesthetized mice using a PIXIImus II (GE Lunar, Madison, WI) densitometer. All experimental mice were scanned at 7, 10, and 15 weeks of age as indicated in Fig. 1A. From the whole body scans, areal bone mineral density (BMD) and bone mineral content (BMC) were calculated for the entire postcranial skeleton using the Lunar ROI tools.

2.3. Micro-computed tomography (μCT)

The right femur was extracted at sacrifice (16 weeks of age) and fixed in 4% PFB for 2 days, then transferred into 70% ethanol. A 2.6-mm span of the distal femoral metaphysis was scanned on a desktop μCT (μCT 20; Scanco Medical AG) at 13-μm resolution using 70-kV peak tube potential and 151-ms integration time to measure trabecular three-dimensional morphometric properties as previously described [21]. Standard trabecular bone parameters (BV/TV, Tb.N, Tb.Th) were calculated from each reconstructed stack through the metaphysis. Cortical thickness (Ct. Th) and area (Ct.Ar) were obtained from 20 slices reconstructed through the midshaft femur at 9-μm resolution.

2.4. Measurements of serum bone resorption markers and osteoclast enumeration in mice

To measure levels of the serum resorption marker carboxy-terminal collagen cross-links (CTX), blood samples were collected from each mouse at 7 weeks of age, allowed to clot for 30 to 60 min, and then centrifuged to separate and collect serum. CTX was measured from the serum using a commercially available plate assay (IDS Ratlaps EIA, Gaithersburg, MD) following the manufacturer's instructions. Serum samples from 10 mice per group were analyzed. Additionally, after conducting μCT measurements on the right femur, we processed those tissues for plastic-embedded thin sectioning and Trap-staining as described previously [22]. Osteoclast number per unit bone surface (Oc.N/BS, #/mm) and osteoclast surface per unit bone surface (Oc.S/BS, %) were measured in metaphyseal cancellous bone.

2.5. Osteoclast culture and osteoclastogenic assays

Bone marrow-derived macrophages (BMMs) were prepared as previously described [23]. Briefly, bone marrow was isolated from 6-week old mice heterozygous for the Lrp5 G171V conditional allele (G171V), with or without the Ctsk-Cre transgene. After culturing for 24 h in α-MEM containing 10% FBS, non-adherent cells were collected and cultured in α-MEM containing 10% FBS with M-CSF and RANKL (PeproTech, NJ, USA) for osteoclastogenesis assays, cells were plated at

Fig. 3. Reduced bone resorption in female, but not male, mice with activated Lrp5 HBM alleles in Ctsk-Cre expressing cells. The resorption marker carboxyl-terminal collagen crosslinks (CTX) was measured from serum collected from (A) female and (D) male Lrp5 heterozygous +/A and +/G mice, with (+) or without (−) the Ctsk-Cre transgene at 7 weeks of age. Osteoclast surface per bone surface (Oc.S/BS; panels B and E) and number of osteoclast per bone surface (N.Oc/BS; panels C and F) were measured in the distal femoral metaphysis in sections from 16-week-old mice by counting tartrate-resistant acid phosphatase (TRAP)-positive cells over bone surface. *p < 0.05 compared to each Cre-negative counterpart, †p < 0.05 compared to +/G Cre-positive mice. Sample size in n = 11/group.
6 × 10⁴ cells/well in a 96 well plate and cultured for 4–5 days until the appearance of multinucleated osteoclasts. The osteoclasts were then fixed in 4% paraformaldehyde for 10 min and then stained for TRAP with 0.1 M acetate solution (pH 5.0) containing 6.7 mM sodium tartrate, 0.12 mg/ml naphthol AS-MX phosphate, and 0.07 mg/ml fast red violet. After TRAP staining, the numbers of red-stained TRAP-positive cells that had more than three nuclei and a red cytosol were counted under a light microscope. The average was calculated from 5 wells of 96-well plates. This counting was repeated five times. To determine the bone resorption activity of osteoclasts, multinucleated osteoclasts cultured in the presence of RANKL and M-CSF for 4–5 days were treated with 0.025% trypsin-EDTA for 3–5 min (with light mechanical scraping) to release the cells. The detached mature osteoclasts were washed in growth media, and an identical number of cells were reseeded onto sterilized cortical bone slices (approx. 200 osteoclasts per slice in 96 well plates; IDS, Ltd., Boldon, UK). After 3 days of culture on bone slices, the conditioned media was collected for biochemical evaluation of osteoclast resorption activity (CTx ELISA, IDS Inc., Gaithersburg, MD). The bone slices were TRAP stained for osteoclast enumeration, and the osteoclast counts were used to normalize the CTx data (CTx/Oc.N). To stain osteoclast resorption pits, the TRAP-stained cells on bone slices were first removed using mechanical agitation, and the slices were incubated with 20 μg/ml of peroxidase-conjugated wheat germ agglutinin for 45 min, followed by the staining of the pits with chromogen 3,3′-diaminobenzidine (Sigma-Aldrich, St Louis, MO). To quantify the resorption on each bone slice, the resorbed pits were identified and measured for area using ImageJ.

2.6. Gene expression of osteoclast-selective transcripts using quantitative PCR

Non-adherent bone marrow cells were prepared as described above and cultured with M-CSF alone to form macrophages (50 ng/ml M-CSF for 3 days) or with M-CSF plus RANKL to form pre-osteoclasts (20 ng/ml M-CSF plus 80 ng/ml RANKL for 3 days) and mature osteoclasts (20 ng/ml M-CSF plus 80 ng/ml RANKL for 5 days). The formation of pre-osteoclasts and mature osteoclasts was confirmed based on their morphology. RNA was isolated from the cell preparations using Qiagen RNeasy kits, and the cDNAs were synthesized from 1 μg of RNA using SuperScript synthesis system (Invitrogen). Quantitative RT-PCR was performed on an ABI 7900HT Real time PCR system (Applied Biosystems) using SYBR Green PCR Master Mix assay (Applied Biosystems) for the following transcripts: Oscar, Destamp, Ctsk, Clcn7, and Atpl6ap1. Primer sequences are listed in Table S1. The amplification reaction was performed for 40 cycles with denaturation at 95 °C for 10 min, followed by annealing at 95 °C for 15 s and extension and detection at 60 °C for 1 min. Gene expression was quantitated using the 2^−ΔΔCT method and normalized to transcripts for the housekeeper...
2.7. Droplet digital PCR assay for genomic recombination of the conditional Lrp5-HBM alleles

Droplet digital PCR (ddPCR) was performed as previously described [24]. Briefly, epiphyseal ends of bone were removed from cleaned long bones and the bone marrow removed by extensive washing with PBS. DNA was extracted from bone pieces using the DNeasy Blood and Tissue Kit (Qiagen) and ~3 ng of cortical bone DNA was used in subsequent PCR reactions. Supermix for Probes mastermix (BioRad, Hercules, CA) was used following the manufacturer’s recommendations. PCR was performed using Eppendorf EP gradient S machines, nanodroplets were created using an automatic droplet generator, amplimer containing droplets were counted with a QX200 sample reader, and data were analyzed using Quantasoft software (all instrumentation from BioRad). The primer pairs and probes described below were purchased from IDT (Coralville, IA) and were used to amplify and quantify the number conditional and recombined alleles. At least 200 amplimer-containing droplets per animal were created in order to measure Cre-mediated recombination. PCR primers, P26-AGTACTGGCTGGCACAGA, P27-CAGGCTGCCCTTGCAGAT, and P28-GTCAGTTTCATAGCCTGA were combined and used in a single PCR reaction to generate a 320-bp amplimer for the conditional allele and a 400-bp amplimer for the recombined allele. The conditional allele was detected using a 5HEX/CCGCAAGCTCTAGAGTCAGCTTCTGAT/3IABkFQ probe, while the recombined allele was detected using a 56-FAM/CGGAATTTAGAGGATCCCCGGGTACC/3IABkFQ probe. PCR parameters were run as follows: 95 °C for 10 min; 94 °C for 30 s, 57 °C for 60 s, 72 °C for 30 s, for 40 cycles at 20% ramp; 98 °C for 10 min, and then 12 °C indefinitely.

2.8. Quantitative histomorphometry

Demeclocycline (60 mg/kg IP) and calcine (12 mg/kg IP) were

Fig. 5. In vitro assessment of transcripts associated with osteoclastogenesis and maturity in macrophages (MQ; day 0 of culture from BMMs), preosteoclasts (day 2 of culture in osteoclast-differentiating conditions), and mature osteoclasts (day 4 in osteoclast-differentiating conditions). Quantitative PCR was used to measure expression of (A) osteoclast associated, immunoglobulin-like receptor (Oscar), (B) Dendrocyte expressed seven transmembrane protein (Dcstamp), (C) Cathespin-K (Csk), (D) Chloride voltage-gated channel 7 (Clcn7), and (E) V-type proton ATPase (Atp6ap1). Expression levels for each gene were normalized to Gapdh expression. *p < 0.05 compared to NTG counterpart. Sample size in n = 3/group.

GAPDH.
injected at 7 and 15 weeks of age, respectively. After collecting left femurs at sacri
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ce (17 weeks of age), the 4% PBF-
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xed femurs were
dehydrated in graded ethanols, cleared in xylene, and embedded in methylmethacrylate (MMA). Thick sections were cut from the midshaft using a diamond-embedded wafering saw. Sections were ground and polished to ~30 μm, mounted and coverslipped, then digitally imaged on a fluorescent microscope. Periosteal and endocortical bone forma-
tion parameters were calculated by measuring the extent of unlabeled perimeter (nL.Pm), single-labeled perimeter (sL.Pm), double-labeled perimeter (dL.Pm), and the area between the double labeling with Image-Pro software (MediaCybernetics Inc., Gaithersburg, MD). The derived histomorphometric parameters mineralizing surface over bone surface (MS/BS), mineral apposition rate (MAR), and bone formation rate (BFR/BS) were calculated using standard procedures described elsewhere [25].

2.9. Statistical methods

Statistical analyses were computed using two-way ANOVA, with Ctsk-Cre genotype, TG(transgenic) or non-NTG (non-transgenic), and Lrp5 genotype, +/A or +/G, as main effects. Significant main effects were followed up with all-pairwise post-hoc comparisons using Fishers protected LSD tests. Significance was taken at p < 0.05. All data are presented as mean ± SEM.

3. Results

3.1. Increased bone mass in mice with activated Lrp5 HBM alleles in Ctsk-Cre expressing cells

To determine changes in bone mineral content and density among mice with Ctsk-Cre-driven activation of Lrp5 HBM alleles, we collected serial whole body DEXA scans from all experimental mice (Fig. 1). Ctsk-Cre TG mice with either Lrp5 HBM allele had increased BMD (5–7% in female, 9–12% in male) and BMC (7–13% in female, 12–15% in male) compared to NTG mice, regardless of sex (Fig. 1). There was no difference in BMC and BMD in male TG mice with the +/A or +/G allele, whereas female TG;+/A mice had significantly higher BMC and BMD than TG;+/G mice (Fig. 1).

Analysis of the distal femur by μCT indicated that male and female TG;+/A and TG;+/G mice had significantly higher trabecular BV/TV, cortical thickness, and cortical area than their NTG littermates (Fig. 2). Vertebral bone mass did not differ between NTG and TG mice for either sex (Fig. S1).

3.2. Serum markers of bone resorption and histological measures of osteoclast prevalence are reduced in female but not male mice with Lrp5 mutations induced in Ctsk-Cre-expressing cells

To evaluate osteoclastic activity in these mice, we measured CTx concentration from serum collected at week 7, and we measured the number of, and surface occupied by, osteoclasts in the distal femur metaphysis after sacri
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ce (Fig. 3). Female TG;+/A and TG;+/G mice had reduced serum CTx concentrations (by 10–13%) compared to their NTG littermates, whereas CTx concentrations did not differ in male TG versus NTG mice. Histomorphometric measurements of osteoclast parameters in 16 wk. old mice were consistent with the serum CTx concentration results; female TG;+/A and TG;+/G mice had significantly fewer osteoclasts in the distal femur, reaching a 13–16% decrease in OCL area. The derived histomorphometric parameters mineralizing surface over bone surface (MS/BS), mineral apposition rate (MAR), and bone formation rate (BFR/BS) were calculated using standard procedures described elsewhere [25].
mice.

3.3. Osteoclast differentiation and activity are reduced in marrow cultures derived from female and not male TG;+/G mice

To determine whether changes in osteoclast-associated measures seen in vivo reflect cell autonomous effects of the active Lrp5 allele, we studied osteoclast differentiation and function in vitro. Bone marrow-derived hematopoietic stem cells from +/G mice, with or without the Ctsk-Cre transgene, were used as a source of OC progenitors, and were induced to differentiate using M-CSF and RANKL. Consistent with what was observed in vivo, TRAP staining indicated 45% fewer OCs differentiated in vitro from bone marrow progenitors recovered female TG;+/- compared to NTG;+/- mice. Also consistent with the sex specific in vivo data, no difference in in vitro OC differentiation was observed between male TG;+/- and NTG mice (Fig. 4A–B). The observed sex-related difference in osteoclast formation was not due to initial seeding density, as the same outcome was observed over three different seeding densities (Fig. S1). Additionally, to measure bone resorbing activity of osteoclasts, we re-plated identical numbers of osteoclast-like cells onto bovine cortical bone slices and measured the resorbed ("pit") area after staining. Bone slices containing TG;+/- osteoclasts from female mice had 60% lower pit area than NTG littermate females, while male-derived preparations showed no detectable difference between groups (Fig. 4C–D). Removal of osteoclasts for re-plating onto the bone slices was not associated with a significant loss of, or damage to, cells from female or male mice (Fig. S3). The amount of CTX released into the culture media was also suppressed in female but not male TG mice (Fig. 4E). Expression of osteoclast-selective genes was reduced in female but not male transgenic mice, compared to respective NTG controls (Fig. 5).

3.4. The Ctsk-Cre transgene recombines conditional Lrp5-HBM alleles in bone cells other than osteoclasts, with greater "off-target" recombination occurring in females rather than males

Although Ctsk-Cre is commonly used to induce Cre-mediated recombination in osteoclasts, it may also be active in other skeletal cell types. These "off-target" effects could contribute to the increased bone mass observed in TG;+/-A and TG;+/-G mice. Because osteocytes express Ctsk, particularly under resorption-inducing conditions (26), and since we previously reported that activation of conditional HBM alleles in osteocytes was sufficient to increase bone mass [5], we determined whether the Ctsk-Cre transgene was active in cortical bone which is enriched for osteocytes. We extracted DNA from femoral cortical bone of TG;+/-G and NTG mice and used ddPCR to measure the percentage of Lrp5 conditional alleles in this tissue that had recombined. The rate of recombination in NTG mice did not exceed the background level for this assay (1%). In contrast, male TG;+/-G mice had ~8% of their cortical bone Lrp5 alleles recombined; the rate of cortical bone Lrp5 recombination in female TG;+/-G mice was even higher (Fig. 6).

In addition to observing off-target expression in osteocytes, we also noted that the Ctsk-Cre TG is active during male gametogenesis. If a sire has the Ctsk-Cre TG and a floxed allele, the allele will be inherited recombined in the offspring. This is why all animals in this study inherited the floxed allele from the sire and the Ctsk-Cre TG from the dam.

3.5. Midshaft femur bone formation parameters are increased on the endocortical but not periosteal surface in mice with Lrp5-HBM mutations induced in Ctsk-Cre-expressing cells

Having found evidence for Ctsk-Cre induced Lrp5 HBM activation in osteocytes, we performed dynamic histomorphometry to assess whether some of the increased bone mass in TG;+/-A and TG;+/-G mice might be due to increased bone formation. Endocortical bone formation (MS/BS and BFR/BS) was significantly increased in male and female TG;+/-A mice compared to NTG controls and increased in male, but not female, TG;+/-G mice compared to controls (Fig. 7). Presence of the TG had no effect on periosteal bone formation.

4. Discussion

The importance LRPS5 signaling to bone anabolism is well known. Mice with global Lrp5 HBM mutations and mice with conditional activation of the Lrp5 HBM allele in osteocytes/osteoblasts, had significantly increased bone formation compared to controls [5,14]. Transgenic mice overexpressing a human LRPS5 G171V cDNA in osteoblasts (G171VCol1a1–Lrp5.G171V) also had significantly increased bone formation [15,27]. LRP5 also appears to have a role in preventing bone catabolism. We reported that Lrp5 HBM mice were protected from the bone catabolic effects of disuse and estrogen deficiency (OVX) [14]. Unknown, was whether the HBM mice were protected from catabolism because of anti-osteoclastogenic signals that originated from osteocytes/osteoblasts, from a cell-autonomous role for LRP5 during osteoclast differentiation/function, or both. Experiments in the present communication were designed to address a cell-autonomous role for LRP5 in osteoclasts.

We observed that Ctsk-Cre-driven recombination of conditional Lrp5 HBM alleles led to increased bone mass and architectural properties in male and female mice (Figs. 1 and 2), which could be due to increased anabolism or decreased catabolism. *In vivo* evidence of decreased catabolism was observed in female, but not male, mice (Fig. 3). Since osteocytes/osteoblasts help regulate osteoclast activity by expressing RANKL and OPG, we performed *in vitro* osteoclast differentiation and activity assays to determine if the LRP5 effects were cell autonomous. We observed significant reductions in the number of osteoclasts that could be differentiated bone marrow precursors in *vitro* from in female (Fig. 4), but not male, TG;+/-G mice, and decreased resorptive ability of isolated TG;+/-G osteoclasts *ex vivo* (Figs. 4 and 5). Taken together, these data are consistent with LRP5 having a cell-autonomous role in osteoclast function; this role appears sex-specific.

Because bone mass was increased in male TG;+/-A and TG;+/-G mice, despite there being no apparent effect on osteoclast differentiation or resorption, we sought another reason these mice developed increased bone mass. We looked for "off target" recombination of the Lrp5 HBM allele in osteocytes, for which pro-anabolic roles of LRP5 are known. Osteocytes express Ctsk, albeit at much lower levels than osteoclasts [26]. We therefore asked whether Ctsk-Cre is also expressed in osteocytes. Droplet digital PCR indicated that Ctsk-Cre was active in ~8 to 17% of osteocytes, with females having greater rates of cortical bone recombination than males. This low, but appreciable, percentage of recombined cells could account for the 2 to 4% increased bone volume fraction in the elevated endocortical bone formation rate seen in TG;+/-A and TG;+/-G mice (Fig. 7). Puzzling, however, is that only endocortical bone formation increased, since global HBM and osteocyte/
osteoblast specific activation of HBM alleles increased endocortical and periosteal bone formation [5]. Therefore, we cannot preclude the possibility that increased endocortical bone formation in Csk-Cre TG mice is the consequence of recombined osteoclasts releasing pro-anabolic coupling factors that affect osteoblasts. The results we obtained using Csk-Cre to activate Lrp5 HBM alleles are not inconsistent with the results obtained by Weivoda et al. [17], who used Csk-Cre to inactivate Lrp5; they found no effect on bone mass. However, these investigators did show that bone mass was reduced when Lrp5 was inactivated using a different and earlier-acting osteoclast Cre driver (Rank-Cre) [17].

In conclusion, our results support the hypothesis that there is a cell-autonomous role for LRP5 in osteoclasts. In female mice, Csk-Cre mediated activation of Lrp5 HBM alleles reduced osteoclast differentiation and bone resoring activity in vivo and in vitro. Csk-Cre mediated activation of Lrp5 HBM alleles also significantly increased bone mass in male mice, however the mechanism is less certain. It is possible that osteoclasts with active Lrp5 HBM alleles released pro-anabolic factors that affected osteoblasts. Alternatively, “off target” Csk-Cre activity in a small percentage of osteocytes may have been sufficient to promote increased endocortical bone formation. Additional experiments are needed to address these two possibilities.

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Disclosure statement

The authors have nothing to disclose.

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