gp130 in late osteoblasts and osteocytes is required for PTH-induced osteoblast differentiation

Therese Standal^{1,3}, Rachelle W Johnson¹, Narelle E McGregor¹, Ingrid J Poulton¹, Patricia W M Ho¹, T John Martin^{1,2} and Natalie A Sims^{1,2}

¹St.Vincent's Institute of Medical Research, 9 Princes St, Fitzroy, Victoria 3065, Australia

²Department of Medicine at St. Vincent's Hospital Melbourne, The University of Melbourne, Fitzroy, Victoria, Australia

³Department of Cancer Research and Molecular Medicine, The KG Jebsen Center for Myeloma Research and Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway

Correspondence
should be addressed
to N A Sims
Email
nsims@svi.edu.au

Abstract

Parathyroid hormone (PTH) treatment stimulates osteoblast differentiation and bone formation, and is the only currently approved anabolic therapy for osteoporosis. In cells of the osteoblast lineage, PTH also stimulates the expression of members of the interleukin 6 (IL-6) cytokine superfamily. Although the similarity of gene targets regulated by these cytokines and PTH suggest cooperative action, the dependence of PTH anabolic action on IL-6 cytokine signaling is unknown. To determine whether cytokine signaling in the osteocyte through glycoprotein 130 (gp130), the common IL-6 superfamily receptor subunit, is required for PTH anabolic action, male mice with conditional gp130 deletion in osteocytes (*Dmp1Cre.gp130^{fl/fl}*) and littermate controls (*Dmp1Cre.gp130^{w/w}*) were treated with hPTH(1–34) (30 µg/kg 5× per week for 5 weeks). PTH dramatically increased bone formation in *Dmp1Cre.gp130^{w/w}* mice, as indicated by elevated osteoblast number, osteoid surface, mineralizing surface, and increased serum N-terminal propeptide of type 1 collagen (P1NP). However, in mice with *Dmp1Cre*-directed deletion of gp130, PTH treatment changed none of these parameters. Impaired PTH anabolic action was associated with a 50% reduction in *Pth1r* mRNA levels in *Dmp1Cre.gp130^{fl/fl}* femora compared with *Dmp1Cre.gp130^{w/w}*. Furthermore, lentiviral-Cre infection of *gp130^{fl/fl}* primary osteoblasts also lowered *Pth1r* mRNA levels to 16% of that observed in infected C57/BL6 cells. In conclusion, osteocytic gp130 is required to maintain PTH1R expression in the osteoblast lineage, and for the stimulation of osteoblast differentiation that occurs in response to PTH.

Key Words

- ▶ glycoprotein-130 (gp130)
- ▶ osteoblast
- ▶ osteocyte
- ▶ osteoclast
- ▶ PTH
- ▶ PTH1R
- ▶ trabecular
- ▶ cortical
- ▶ bone formation

Journal of Endocrinology
(2014) 223, 181–190

Introduction

Intermittent administration of parathyroid hormone (PTH) to animal models and humans (teriparatide (Forteo)) increases bone mass (Reeve *et al.* 1980, Neer *et al.* 2001, Lindsay *et al.* 2007), and is the only approved treatment for osteoporosis capable of inducing bone

formation (reviewed in Hodsman *et al.* (2005) and Khosla *et al.* (2008)). However, the mechanisms by which intermittent PTH increases bone mass remain unclear, and identifying downstream targets of this pathway may aid in the design of improved anabolic therapies.

The effects of PTH on bone mass are likely to be mediated by cells of the osteoblast lineage. This lineage includes committed pre-osteoblasts, matrix-producing osteoblasts, bone lining cells, and matrix-embedded osteocytes. PTH acts directly at each stage of differentiation, as follows. PTH promotes pre-osteoblast differentiation (Dobnig & Turner 1995), inhibits osteoblast apoptosis (Jilka *et al.* 1999), and reactivates quiescent lining cells to become active osteoblasts (Kim *et al.* 2012). PTH also acts directly on osteocytes to reduce their expression of the WNT antagonist sclerostin, an inhibitor of bone formation (Bellido *et al.* 2005, Keller & Kneissel 2005).

PTH also stimulates the expression of receptor activator of NF-kappa-B ligand (RANKL) by early osteoblast lineage cells, thereby promoting osteoclast differentiation (Udagawa *et al.* 1999). However, the stages of osteoblast differentiation most important for the actions of PTH remain controversial, because the expression of RANKL by matrix-embedded osteocytes is also stimulated by PTH (Xiong *et al.* 2011).

PTH also acts on the osteoblast lineage to rapidly promote the transcription of interleukin 6 (IL-6) family cytokines and receptors. These include *Il6* (Greenfield *et al.* 1996), *Il11*, oncostatin M receptor (*Osmr*), leukemia inhibitory factor (*Lif*), and cytokine receptor-like factor 1 (*Crlf1*) (Walker *et al.* 2012). These cytokines all depend on the promiscuous co-receptor glycoprotein 130 (gp130) for signaling (reviewed in Sims & Walsh (2010)), and gp130 expression by the osteoblast lineage is also stimulated by PTH (Romas *et al.* 1996).

Many of the actions and gene targets of IL-6 family cytokines are common to those of PTH. As is the case with PTH, the cytokines IL-6, IL-11, OSM, LIF, and cardiotrophin (CT-1) promote osteoblast differentiation *in vitro* (Walker *et al.* 2008, 2010) and OSM, LIF, and CT-1 stimulate bone formation *in vivo* (Cornish *et al.* 1993, Walker *et al.* 2008, 2010). The family members IL-11, LIF, OSM, CT-1, and CNTF also inhibit osteocytic sclerostin expression (Walker *et al.* 2010, Johnson *et al.* 2014a). In addition, IL-6, IL-11, OSM, LIF, and CT-1, stimulate osteoblast lineage expression of RANKL (O'Brien *et al.* 1999, Palmqvist *et al.* 2002, Walker *et al.* 2008) and promote osteoclastogenesis when precursors are co-cultured with osteoblasts *in vitro* (Tamura *et al.* 1993, Richards *et al.* 2000). These similar effects and the upregulation of IL-6 family cytokines in osteoblasts by PTH suggest that this cytokine family may play a role in the actions of PTH on the osteoblast lineage.

Hence, in this study we examined the requirement of gp130 signaling in osteocytes for the anabolic action of

PTH, using mice with *Dmp1Cre*-directed deletion of gp130 in osteocytes (*Dmp1Cre.gp130^{ff}*; Johnson *et al.* 2014b) and mature osteoblasts (Xiong *et al.* 2011, Torreggiani *et al.* 2013). We found that gp130 in these cells is required for PTH to increase osteoblast number and bone forming surfaces, and to maintain PTH1R expression in the osteoblast lineage.

Materials and methods

Mice

All animal procedures were conducted with the approval of the St. Vincent's Health Melbourne Animal Ethics Committee. *Dmp1Cre* mice were obtained from Lynda Bonewald (University of Kansas, Kansas City, MO, USA; Lu *et al.* 2007). Floxed gp130 mice backcrossed onto C57/BL6 were obtained from Rodger McEver (Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; Betz *et al.* 1998). Mice hemizygous for the *Cre* transgene were crossed with the gp130 flox mouse in which the transmembrane domain (exon 15) was flanked by loxP sites, resulting in ablation of intracellular gp130 signaling, as previously reported (Betz *et al.* 1998) and confirmed at the mRNA level in bone (Johnson *et al.* 2014b). For all experiments, *Dmp1.Cre*+ cousins were used as controls.

Six-week-old male *Dmp1Cre.gp130^{w/w}* or floxed *Dmp1Cre.gp130^{ff}* mice were injected i.p. with 30 µg/kg human PTH 1–34 (hPTH 1–34) or vehicle, 5 days a week for 5 weeks (*n*=9/10 per group). This dose and duration of PTH treatment were chosen because it provides a robust increase in lamellar bone formation rate and osteoblast surface in male mice without increasing osteoclastogenesis (Walker *et al.* 2012, Takyar *et al.* 2013, Tonna *et al.* 2014). The mice were also injected with calcein (20 mg/kg) 7 and 2 days before tissue collection. The bones were collected 1 h after the last PTH injection. The mice were fasted for 12 h before anaesthesia with ketamine/xylazine and a final blood sample was collected by cardiac puncture. The blood samples were centrifuged for 10 min at 4000 g and the serum was collected in a fresh tube and stored at –80 °C until analysis for cross-linked C-terminal telopeptide of type 1 collagen (CTX1), N-terminal propeptide of type 1 collagen (P1NP) (Immunodiagnostic Systems Limited, Boldon, Tyne and Wear, UK), and PTH (Immunotopics, San Clemente, CA, USA) as per manufacturer's instructions. One femur was flushed of marrow and the bone shaft was collected for RNA analyses as described previously (Walker *et al.* 2012). Briefly, bones were homogenized with a LS-10-35 Polytron homogenizer in

TRI for 4×5 s bursts and stored at −80 °C. RNA from each bone was purified using the RNeasy lipid tissue minikit (Qiagen), according to manufacturer's instructions.

The other femur was analyzed by micro-computed tomography as described previously (Johnson *et al.* 2014b) using the SkyScan 1076 System (Bruker-microCT, Kontich, Belgium). The images were acquired using the following settings: 9 µm voxel resolution, 0.5 mm aluminium filter, 48 kV voltage, and 100 µA current, exposure time, rotation 0.5°, frame averaging=1. The images were reconstructed and analyzed using SkyScan Software programs NRecon (version 1.6.3.3), DataViewer (version 1.4.4), and CT Analyser (version 1.12.0.0). Femoral trabecular analysis region of interest (ROI) was determined by identifying the distal end of the femur and calculating 15% of the total femur length toward the femora mid-shaft, where we then analyzed an ROI of 12.6% of the total femur length. The analysis of bone structure was completed using adaptive thresholding (mean of min and max values) in CT Analyser. The thresholds for analysis were determined based on multilevel Otsu thresholding of the entire data set, and were set at 45–255 for trabecular bone. The cortical analyses were performed at 35% above the distal end of the femur toward the femora mid-shaft, also with a 12.6% ROI with the threshold values set at 100–255.

Tibiae were collected for histomorphometric analyses as previously described (Sims *et al.* 2006). Briefly, trabecular histomorphometry was carried out on undecalcified sections in the secondary spongiosa of the proximal tibia, in a region 370 µm below the proximal edge of the hypertrophic zone of the growth plate, extending 1.11 mm in the proximal direction. Periosteal histomorphometry was carried out on the antero-fibular side of the tibia, commencing 1.11 mm below the chondro-osseous junction of the growth plate, and extending 1.11 mm in the proximal direction. The nomenclature is as described previously (Parfitt *et al.* 1987).

Lenti-Cre viral infection

Calvarial osteoblasts were collected from C57/BL6 WT and *gp130^{fl/fl}* neonates by digesting calvaria in 1:2 collagenase II/dispase solution at 37 °C on a shaker (1×5 min 4×10 min digestions). The cells were resuspended in culture media (alpha-MEM+10% fetal bovine serum), and allowed to adhere overnight before being frozen and stored in liquid nitrogen. When required, isolated cells were thawed and expanded in culture and infected with a GFP-tagged lenti-Cre virus synthesized as described previously (Tonna *et al.* 2014) for 24 h with polybrene in

the maintenance media. Following infection, media was changed and cells were evaluated for GFP expression by microscopy; >30–60% transfection efficiency was observed (*n*=3 independent experiments). The cells were expanded in culture for 2–3 weeks in alpha-MEM+10% fetal bovine serum, and GFP positive cells (fluorescence driven by *Cre* transgene expression) were sorted on a FACS Aria (BD Biosciences, San Jose, CA, USA) for GFP. The GFP+ cells were harvested for RNA in trizol (Life Technologies) and separated and precipitated using chloroform and isopropanol. Extracted RNA was treated with DNase using Ambion TURBO DNA-free Kit (Life Technologies) and quantified on a NanoDrop ND1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Semi-quantitative real-time PCR

cDNA synthesis from 50 to 100 ng DNase-treated RNA from each femur or cell culture preparation was carried out using AffinityScript (Agilent Technologies, Santa Clara, CA, USA) as per the manufacturer's instructions. The stock cDNA was diluted to a concentration of 5 ng/µl and semi-quantitative real-time PCR (qPCR) was performed on 12.5 ng cDNA in a reaction volume of 10 µl using in-house master mix of 10× AmpliTaq Gold with SYBR Green nucleic acid gel stain (Life Technologies). *Dkk1* primers were designed using NCBI Primer Blast: forward, GAG-GGGAAATTGAGGAAAGC and reverse, ACGGAGC-CTTCTTGTCCTTT. Other primers were as previously described for *Pth1r*, hypoxanthine phosphoribosyltransferase 1 (*Hprt1*), *Sost*, *Tnfrsf11*, *Il6* (Allan *et al.* 2008), β-2 microglobulin (*B2m*) (McGregor *et al.* 2010), and hydroxymethylbilane synthase (*Hmbs*) (Johnson *et al.* 2014b).

The samples were dispensed onto optically clear 96-well plates (Thermo Scientific) and run on a Stratagene Mx3000P (Agilent Technologies). The cycling conditions were 95 °C for 10 min (95 °C for 30 s, 58 °C for 1 min, and 72 °C for 30 s)×40 cycles, followed by a dissociation step (95 °C for 1 min, 55 °C for 30 s, and 95 °C for 30 s). The post-run samples were analyzed using MxPro (Agilent Technologies, Santa Clara, CA, USA) and reported using linear ΔCT values normalized to the geometric mean of the two housekeeping genes (HKG) *Hprt1* and *Hmbs* or to *B2m* as indicated.

Statistical analysis

All graphs are presented as the mean/genotype+s.e.m.. *N*=5–10 animals/group as indicated in the figure legend. For *in vitro* experiments, data shown is the average of three

independent biological replicates. Statistical significance was considered when $P < 0.05$. Differences between groups were analyzed by two-way ANOVA and *post hoc* Sidak multiple comparison test. Skewed variables were transformed using the natural logarithm before statistical analyses. For the lenti-viral *Cre*-infected primary calvarial osteoblasts, Student's *t*-test was used to assess significance. Statistical analyses were performed using GraphPad Prism version 6.0c for Mac OS X (GraphPad Software, La Jolla, CA, USA).

Results

Dmp1Cre.gp130^{ff} mice show no increase in the number of trabecular osteoblasts in response to PTH

PTH treatment at 30 $\mu\text{g/kg}$ per day significantly increased osteoblast number/bone perimeter (NOB/BPm) on trabecular bone in *Dmp1Cre.gp130^{w/w}* mice by 76% (Fig. 1A). Osteoblast surface/bone surface (Obs/BS; Fig. 1B) and osteoid surface/bone surface (OS/BS; Fig. 1C) were also elevated by PTH treatment to similar extents. We detected no significant changes in osteoid thickness in *Dmp1Cre.gp130^{w/w}* mice after PTH treatment (Fig. 1D).

In contrast to *Dmp1Cre.gp130^{w/w}* mice, PTH treatment did not increase osteoblast or osteoid-derived parameters in age- and sex-matched *Dmp1Cre.gp130^{ff}* mice (Fig. 1A, B, C and D). Two-way ANOVA revealed that the effects of PTH treatment on both NOB/BPm and Obs/BS were significantly reduced in the *Dmp1Cre.gp130^{ff}* mice compared with *Dmp1Cre.gp130^{w/w}* controls (interaction $P = 0.039$ and $P = 0.043$ respectively). This indicates that the effect of PTH on osteoblast differentiation is dependent on gp130 expression in osteocytes.

In line with the effects on osteoblast numbers, bone forming surfaces, indicated by incorporation of calcein labels, including both double-labeled surface (dLS/BS) (Fig. 1E) and single-labeled surface ($P \leq 0.05$, not shown) were significantly greater in PTH-treated *Dmp1Cre.gp130^{w/w}* mice compared with controls. Again, this was not observed in *Dmp1Cre.gp130^{ff}* mice. Mineral apposition rate (MAR) was significantly greater in both *Dmp1Cre.gp130^{ff}* and *Dmp1Cre.gp130^{w/w}* mice treated with PTH compared with their vehicle-treated controls (Fig. 1F), indicating that an increase in mineralization rate in response to PTH is retained on those surfaces on which bone formation occurs in *Dmp1Cre.gp130^{ff}* mice.

PTH-treated *Dmp1Cre.gp130^{w/w}* mice had significantly higher serum P1NP levels than *Dmp1Cre.gp130^{w/w}* untreated controls. In contrast, in *Dmp1Cre.gp130^{ff}* mice there was no significant effect of PTH on P1NP levels

Figure 1

Osteocytic gp130 is required for PTH to increase osteoblast numbers and bone formation in trabecular bone. Male mice were treated with hPTH (1–34) at 30 $\mu\text{g/kg}$ per day for 5 weeks. (A) Numbers of osteoblasts/bone perimeter (NOB/BPm), (B) osteoblast surface/bone surface (Obs/BS), (C) osteoid surface/bone surface (OS/BS), (D) osteoid thickness (OTh), (E) double calcein-labeled surface (dLS/BS), and (F) mineral apposition rate (MAR) from trabecular bone in the proximal tibial secondary spongiosa in *Dmp1Cre.gp130^{w/w}* (gp130 w/w) and *Dmp1Cre.gp130^{ff}* (gp130 ff) mice. (G) The serum levels of procollagen type 1 amino-terminal propeptide (P1NP) and (H) endogenous murine PTH measured at the end of the treatment protocol are also shown. Scale bars are mean $\pm$ S.E.M., $n = 8–10$ per group. * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$, PTH-treated compared with genotype-matched vehicle-treated mice; ++ $P < 0.01$ compared to vehicle-treated gp130 w/w mice.

compared with vehicle-treated *Dmp1Cre.gp130^{ff}* mice (Fig. 1G); interaction P value = 0.009 by two-way ANOVA. These results are consistent with the histomorphometry data and confirm that at a systemic level, the effect of PTH on bone formation is blunted in *Dmp1Cre.gp130^{ff}* mice.

In both *Dmp1Cre.gp130^{ff}* and *Dmp1Cre.gp130^{w/w}* mice, intermittent human PTH treatment led to reduced production of endogenous circulating murine PTH levels (Fig. 1H), demonstrating that negative feedback at the parathyroid gland induced by exogenous PTH administration was maintained in both groups of mice.

Table 1 Effects of PTH on trabecular and cortical bone in femora from *Dmp1Cre.gp130^{w/w}* and *Dmp1Cre.gp130^{w/w}* mice.

	<i>Dmp1Cre.gp130^{w/w}</i>		<i>Dmp1Cre.gp130^{f/f}</i>	
	Vehicle (n=9)	PTH (n=10)	Vehicle (n=9)	PTH (n=9)
BV/TV (%)	24.86 ± 0.42	21.54 ± 1.32	17.70 ± 1.55 ⁺⁺⁺	20.61 ± 0.96
Tb.Th (μm)	57.61 ± 1.80	56.49 ± 2.22	57.92 ± 3.01	57.42 ± 3.83
Tb.N (/mm)	4.35 ± 0.15	3.80 ± 0.16	3.03 ± 0.15 ⁺⁺⁺	3.64 ± 0.18
Tb.Sp (μm)	122.71 ± 2.96	144.39 ± 14.04	171.05 ± 15.74 ⁺⁺⁺	158.98 ± 10.37
Ct.Ar (mm ²)	0.60 ± 0.02	0.65 ± 0.02	0.63 ± 0.02	0.67 ± 0.03

Fixed nondemineralized femora from vehicle- or PTH-treated mice were analyzed by μ CT. Effect of *gp130^{f/f}* transgene: ⁺⁺⁺ $P < 0.001$ vs *Dmp1Cre.gp130^{w/w}* (two-way ANOVA with Sidak multiple comparisons test). BV/TV, bone volume per total volume of the region of interest; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Ct.Ar, cortical area.

Although this dose of PTH significantly increased all markers of bone formation in *Dmp1Cre.gp130^{w/w}* mice, we did not detect a significant increase in trabecular bone mass by micro-computed tomography with this short-time course of low-dose treatment (Table 1). The low trabecular bone mass of these mice, previously reported (Johnson *et al.* 2014b), was confirmed.

No effect of intermittent PTH treatment on bone resorption

This protocol of intermittent PTH treatment did not significantly change osteoclast number/bone perimeter (NOc/BPm) (Fig. 2A), osteoclast number/osteoclast perimeter (Fig. 2B), osteoclast surface/bone surface (OcS/BS) (Fig. 2C), or serum levels of cross-linked CTX1 (Fig. 2D) in either *Dmp1Cre.gp130^{f/f}* or *Dmp1Cre.gp130^{w/w}* mice. This confirms our previous observations using similar protocols over 4 weeks of treatment (Walker *et al.* 2012, Takyar *et al.* 2013, Tonna *et al.* 2014).

Effects of PTH on cortical bone

Periosteal MAR (Fig. 3A), periosteal mineralising surface (Fig. 3B) and periosteal perimeter (Fig. 3C) were all significantly greater in PTH-treated *Dmp1Cre.gp130^{w/w}* mice compared with untreated mice. None of these parameters were significantly increased by PTH treatment in *Dmp1Cre.gp130^{f/f}* mice compared with genotype-matched vehicle controls (Fig. 3A–C), indicating that periosteal growth in response to PTH may also be impaired in the absence of osteocytic *gp130*.

Normal response of osteoclastic genes, but lack of inhibition of WNT signaling inhibitors by PTH treatment in *Dmp1Cre.gp130^{f/f}* mice

RANKL (gene name *Tnfsf11*) and IL-6 (*Il6*) are both potent stimuli of osteoclast formation, and PTH increases their

expression in cells of the osteoblast lineage (Greenfield *et al.* 1995, Udagawa *et al.* 1999). Indeed, in marrow-flushed femoral samples collected 1 h after the last of these 5 weeks of injections, mRNA levels of *Tnfsf11* and *Il6* were significantly higher in both genotypes after PTH treatment (Fig. 4A and B); this increase was not significantly affected by the genotype (two-way ANOVA interaction P values = 0.365 and 0.314 respectively). This indicated that among cells in the flushed femora, which would include osteoblasts at different stages of differentiation as well as osteocytes, are some cells that retain normal responses of these genes to PTH.

Wingless (WNT)-signaling is important for osteoblast differentiation and bone formation, and PTH has been shown to stimulate WNT signaling by suppressing Dickkopf1 (*Dkk1*) and sclerostin (*Sost*) expression in the

Figure 2

No effect of intermittent PTH on bone resorption. Male mice were treated with hPTH (1–34) at 30 μ g/kg per day for 5 weeks. (A) Numbers of osteoclasts per unit bone perimeter (NOc/BPm), (B) numbers of osteoclasts per unit osteoclast perimeter (NOc/OcPm), (C) osteoclast surface per unit bone surface (OcS/BS) measured in the proximal tibial secondary spongiosa, and (D) serum levels of cross-linked C-terminal telopeptide of type 1 collagen (CTX1) in PTH and vehicle-treated *Dmp1Cre.gp130^{w/w}* (gp130 w/w) and *Dmp1Cre.gp130^{f/f}* (gp130 f/f) mice. Scale bars are mean $\pm$ s.e.m., $n = 8–10$ per group.

Figure 3

PTH effects on cortical bone are impaired in *Dmp1Cre.gp130^{fl/fl}* mice. Male mice were treated with hPTH (1–34) at 30 μg/kg per day for 5 weeks. (A) Tibial periosteal MAR (Ps.MAR), and (B) mineralizing surface per unit bone surface (MS/BS) in the tibial diaphysis, and (C) periosteal perimeter (Ps.Pm) of the femoral diaphysis in PTH and vehicle-treated *Dmp1Cre.gp130^{w/w}* (gp130 w/w) and *Dmp1Cre.gp130^{fl/fl}* (gp130 f/f) mice. Scale bars are mean ± s.e.m., n = 8–10 per group. **P ≤ 0.01, NS, P > 0.05 (not statistically significant) in PTH-treated compared with genotype-matched vehicle-treated mice. +, P < 0.05, vehicle-treated *Dmp1Cre.gp130^{fl/fl}* compared with vehicle-treated *Dmp1Cre.gp130^{w/w}*.

osteoblast lineage (Keller & Kneissel 2005, Yao *et al.* 2011). For this reason, we quantified mRNA levels of *Dkk1* and *Sost* in flushed femurs. As expected, *Dkk1* mRNA levels were significantly lower in PTH-treated *Dmp1Cre.gp130^{w/w}* femurs compared with untreated mice. However, *Dkk1* was not lower in femurs from PTH-treated *Dmp1Cre.gp130^{fl/fl}* mice compared with controls (Fig. 4C). *Sost* mRNA levels were slightly, but not significantly, lowered in response to PTH in *Dmp1Cre.gp130^{w/w}* femora. *Dmp1Cre.gp130^{fl/fl}* femora showed a lower level of *Sost* mRNA compared with vehicle-treated *Dmp1Cre.gp130^{w/w}* controls; with PTH treatment these mice showed a

significant increase in *Sost* mRNA levels (Fig. 4D). These differences in the effects of PTH treatment on gene expression were significant by two-way ANOVA for both *Dkk1* (interaction P = 0.01) and *Sost* (interaction P = 0.003). Thus, PTH treatment does not decrease WNT antagonist expression in *Dmp1Cre.gp130^{fl/fl}* mice, implying that gp130 signaling in osteocytes is important for the PTH effect on WNT signaling inhibitors.

PTH1r expression is reduced in *DMP1Cre.gp130^{fl/fl}* mice and gp130-deficient osteoblasts

Since many effects of PTH were blocked in *Dmp1Cre.gp130^{fl/fl}* mice, we quantified *Pth1r* mRNA levels in flushed femurs from untreated 12-week-old *Dmp1Cre.gp130^{fl/fl}* and *Dmp1Cre.gp130^{w/w}* mice. Surprisingly, *Pth1r* mRNA expression was 47% lower in *Dmp1Cre.gp130^{fl/fl}* compared with *Dmp1Cre.gp130^{w/w}* femurs (P = 0.03; Fig. 5A).

These findings were supported by *in vitro* data, where C57/BL6 and *gp130^{fl/fl}* calvarial osteoblasts were infected with lentiviral *Cre*-recombinase. In *Cre*-infected *gp130^{fl/fl}* osteoblasts, *gp130* was significantly lowered by 52%, and *Pth1r* mRNA was 84% lower than in infected C57/BL6 cells

Figure 4

PTH effects on WNT-inhibitor, but not osteoclastogenic, mRNA levels are impaired in *DMP1Cre.gp130^{fl/fl}* mice. RNA was isolated from femurs flushed of bone marrow and expression of PTH target genes was examined by relative quantitative PCR. *Tnfrsf11* mRNA (A), *Il6* mRNA (B), *Dkk1* mRNA (C) and *Sost* mRNA (D) in *Dmp1Cre.gp130^{w/w}* and *Dmp1Cre.gp130^{fl/fl}* mice treated for 5 weeks with PTH, collected 1 h after the final injection. All values are shown relative to housekeeping (Hkg) – the geometric mean of hypoxanthine phosphoribosyltransferase 1 (*Hprt1*) and hydroxymethylbilane synthase (*Hmbs*). Scale bars are mean ± s.e.m., n = 5–8 bones per group, with mRNA prepared and analyzed separately. **P ≤ 0.01 and ***P ≤ 0.001, PTH-treated compared with genotype-matched vehicle-treated mice; +P < 0.05, vehicle-treated *Dmp1Cre.gp130^{fl/fl}* compared with vehicle-treated *Dmp1Cre.gp130^{w/w}*.

Figure 5

PTH1R expression is reduced in *DMP1Cre.gp130^{ff}* mice and gp130 deficient cultured osteoblasts. (A) *Pth1r* mRNA quantified by qPCR in femurs flushed of bone marrow obtained from untreated 12-week-old *Dmp1Cre.gp130^{w/w}* and *Dmp1Cre.gp130^{ff}* mice, normalized to *Hmbs*; *n*=8 samples per group. (B) *gp130* (*Il6st*) and (C) *Pth1r*, *Runx2*, *Osx* and *Alpl* mRNA levels in primary calvarial osteoblasts obtained from *gp130^{ff}* or C57/BL6 WT neonates infected with lentiviral Cre-recombinase; levels are shown normalized to beta-2-microglobulin (*B2m*) (*n*=3 biological replicates). **P*≤ 0.05; ***P*≤0.01, vs *gp130 w/w* or C57/BL6.

(Fig. 5B and C). The mRNA levels of *Runx2*, *Osx*, and *Alpl* were not significantly altered by Cre-infection of *gp130^{ff}* osteoblasts (Fig. 5C), consistent with previously published mRNA levels of these genes in the femora of *Dmp1Cre.gp130^{ff}* mice (Johnson *et al.* 2014b). This suggests that the cells of the osteoblast lineage require signals mediated by gp130 to maintain PTH1R expression, and that a lack of PTH1R in *Dmp1Cre* expressing cells is responsible for the reduced response to anabolic PTH treatment.

Discussion

This work demonstrates that PTH-induced osteoblast differentiation is dependent on gp130 expression in mature osteoblast lineage cells. gp130 is needed to maintain *Pth1r* expression in osteoblasts, and is required for PTH to suppress the WNT-antagonists *Dkk1* and *Sost*. In contrast, gp130 expression by osteocytes is not required for PTH to stimulate mRNA levels of the pro-osteoclastogenic factors RANKL (*Tnfsf11*) and *Il6* in bone.

The stimulatory effect of PTH on trabecular osteoblast numbers and mineralizing surface was completely ablated in *Dmp1Cre.gp130^{ff}* mice. This may, at least partly, be explained by the lack of a reduction in both WNT signaling inhibitors *Sost* and *Dkk1* in response to PTH. WNT signaling stimulates osteoblast differentiation, and it has been postulated that this is one pathway through which PTH stimulates bone formation (Kulkarni *et al.* 2005), a hypothesis supported by impaired PTH responses in mice overexpressing sclerostin or *Dkk1* (Guo *et al.* 2010, Kramer *et al.* 2010). PTH directly inhibits *Sost* via cAMP-PKA signaling (Keller & Kneissel 2005). The IL-6 family cytokines also rapidly inhibit *Sost*, although the mechanism remains unknown (Walker *et al.* 2010). Whether the reduction in the effect of PTH on WNT signaling is entirely due to the reduced PTH1R expression or results from some dependence on gp130 cytokines by this same pathway in osteoblasts and osteocytes remains unclear.

In contrast to the effect on WNT-antagonists, both *Dmp1Cre.gp130^{w/w}* and *Dmp1Cre.gp130^{ff}* mice demonstrated increased femoral *Tnfsf11* and *Il6* mRNA levels in response to PTH. Despite these increases in both genotypes, osteoclast numbers were unchanged, as we have previously reported with this low dose of intermittent PTH treatment (Takyar *et al.* 2013, Tonna *et al.* 2014), likely because the inductions of *Tnfsf11* and *Il6* are transient (Ma *et al.* 2001, Walker *et al.* 2012). IL-6 and RANKL are expressed by a wide range of cells in the bone, including osteoblast lineage cells as well as osteocytes (Lee & Lorenzo 1999, Dai *et al.* 2006, Nakashima *et al.* 2011, Xiong *et al.* 2011), and cells within the bone marrow, including T-cells (Horwood *et al.* 1999, Hirano *et al.* 1986) and, in the case of IL-6, macrophages (Tosato *et al.* 1988). Although PTH has recently been suggested to directly promote RANKL expression in osteocytes (Xiong *et al.* 2011), our findings suggest that the major cellular targets that produce these pro-osteoclastogenic factors in response to PTH are not osteocytes. Notably, although PTH was unable to increase osteoblast numbers or mineralizing surface in the *Dmp1Cre.gp130^{ff}* mice, on those surfaces where double calcein labels were incorporated into the bone matrix, the distance between them (MAR) was significantly greater in PTH-treated mice, regardless of genotype. This suggests that bone-forming osteoblasts in *Dmp1Cre.gp130^{ff}* mice retain sufficient PTH1R expression to respond to PTH with increased matrix production. Since marrow was flushed from the femora, and *Pth1r* levels were dramatically reduced in undifferentiated cultured Cre-expressing cells, we suggest that the

key PTH-responsive cells producing RANKL and IL-6 in this model are less differentiated osteoblasts, not expressing DMP1Cre, on the bone surface.

Pth1r mRNA was lower in cortical bone of *Dmp1Cre.gp130^{ff}* mice compared with littermate controls, an effect that was reproduced when gp130 was deleted in cultured primary calvarial osteoblasts. There are two ways to understand this: firstly, as osteoblast differentiation is impaired in the *Dmp1Cre.gp130^{ff}* mice (Johnson *et al.* 2014b) and PTH1R expression in the osteoblast lineage is higher in more mature osteoblasts (Allan *et al.* 2003, 2008, Balic *et al.* 2010), there may be fewer mature PTH1R-expressing osteoblasts present within the bone of these mice. Another interpretation is that gp130 is needed to maintain the expression of PTH1R in the osteoblast lineage. This latter hypothesis is supported by our *in vitro* data, as we observed that a reduction in gp130 by about 50% in calvarial osteoblasts cultured *in vitro* reduced *Pth1r* mRNA levels by nearly 80%. This further suggests that, as well as maintaining PTH1R levels in the osteocyte, gp130 may maintain PTH1R expression throughout the osteoblast lineage.

Although *Pth1r* levels were low in the femora of *Dmp1Cre.gp130^{ff}* mice, their phenotype is strikingly different to mice with a conditional deletion of *Pth1r* in osteocytes (Ocy-PPRKO), generated using the same *Dmp1Cre* (Saini *et al.* 2013). Ocy-PPRKO mice showed a greater trabecular bone mass than controls, with no significant alteration in osteoblast numbers, indicating that the underlying cause of bone fragility in the *Dmp1Cre.gp130^{ff}* mice is not simply low PTH1R expression in the osteocyte. As observed in *Dmp1Cre.gp130^{ff}* mice, Ocy-PPRKO mice failed to reduce *Sost* in response to PTH treatment. However, in direct contrast to *Dmp1Cre.gp130^{ff}* mice, Ocy-PPRKO mice lacked a *Tnfsf11* in response to PTH. This suggests that the *Dkk1/Sost* and *Tnfsf11/Il6st* responses to PTH occur in different cell populations, and it is only the former that is affected by *Dmp1Cre*-mediated gp130 deletion. Alternatively, the *Dkk1/Sost* induction may require a higher level of PTH1R expression than the *Tnfsf11/Il6st* response; the low level of PTH1R expression in the *Dmp1Cre.gp130^{ff}* mice may be sufficient for the latter.

In addition to mediating the response of osteoblasts to exogenous PTH treatment, PTH1R also acts as a receptor for PTH-related protein (PTHrP). Although first identified as the mediator of humoral hypercalcemia of malignancy (Suva *et al.* 1987), PTHrP is also produced by the osteoblast lineage (Kartsogiannis *et al.* 1997). This local PTHrP production is essential for normal osteoblast

differentiation, as indicated by studies of an osteoblast-lineage PTHrP-null mice (Miao *et al.* 2005). This suggests that basal defects in osteoblast differentiation in our model lacking gp130 in osteocytes may relate specifically to a lack of PTHrP signal. Notably, and in direct contrast to our model, the osteoblast-lineage knockout of PTHrP also exhibited a significant impairment in osteoclastogenesis (Miao *et al.* 2005), a finding that may relate to the difference in the gene-driving expression of the *Cre*-recombinase. The *Pthrp^{ff}* deletion was driven by the *Col2.3Cre*, which would delete expression in osteocytes, but also in less mature osteoblasts than the *Dmp1Cre* that we have used. Again, this suggests that the PTH-induced expression of RANKL is likely to occur in less mature osteoblasts.

In conclusion, in addition to the recently described role of osteocytic gp130 in maintaining bone formation and strength (Johnson *et al.* 2014b), the current study has revealed a new role for gp130 in the osteoblast lineage in bone: it is needed to maintain PTH1R expression and to increase osteoblast numbers in response to anabolic PTH treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by a National Health and Medical Research Council (Australia) Project Grant 1002728. N A S is supported by a National Health and Medical Research Council (Australia) Senior Research Fellowship. T S is supported by a Senior Research Fellowship from the Norwegian Cancer Society (project number 538153). The Victorian State Government Operational Infrastructure Support Scheme provides support to St. Vincent's Institute of Medical Research.

Author contributions

N A S and T S designed the study; T S, R W J, N E M, I J P, and P W M H conducted the study and made data collection; T S, R W J, N E M, I J P, P W M H, and N A S analyzed data; T S, R W J, N E M, I J P, P W M H, T J M, and N A S interpreted data; T S and N A S drafted manuscript; T S, N A S, R W J, and T J M revised the manuscript content. All authors approved the final version of manuscript. N A S takes responsibility for the integrity of the data analysis.

Acknowledgements

The authors thank Joshua Johnson for technical expertise in the processing of bone samples for histomorphometric analysis, Dr Stephen Tonna for advice on Lentiviral *Cre* infection, and Dr Nicole Walsh for design of the *Dkk1* primers.

References

- Allan EH, Ho PW, Umezawa A, Hata J, Makishima F, Gillespie MT & Martin TJ 2003 Differentiation potential of a mouse bone marrow stromal cell line. *Journal of Cellular Biochemistry* **90** 158–169. (doi:10.1002/jcb.10614)
- Allan EH, Hausler KD, Wei T, Gooi JH, Quinn JM, Crimeen-Irwin B, Pompolo S, Sims NA, Gillespie MT, Onyia JE *et al.* 2008 EphrinB2 regulation by PTH and PTHrP revealed by molecular profiling in differentiating osteoblasts. *Journal of Bone and Mineral Research* **23** 1170–1181. (doi:10.1359/jbmr.080324)
- Balic A, Aguila HL & Mina M 2010 Identification of cells at early and late stages of polarization during odontoblast differentiation using pOB-Col3.6GFP and pOBCol2.3GFP transgenic mice. *Bone* **47** 948–958. (doi:10.1016/j.bone.2010.08.009)
- Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, Manolagas SC & Jilka RL 2005 Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology* **146** 4577–4583. (doi:10.1210/en.2005-0239)
- Betz UA, Bloch W, van den Broek M, Yoshida K, Taga T, Kishimoto T, Addicks K, Rajewsky K & Muller W 1998 Postnatally induced inactivation of gp130 in mice results in neurological, cardiac, hematopoietic, immunological, hepatic, and pulmonary defects. *Journal of Experimental Medicine* **188** 1955–1965. (doi:10.1084/jem.188.10.1955)
- Cornish J, Callon K, King A, Edgar S & Reid IR 1993 The effect of leukemia inhibitory factor on bone *in vivo*. *Endocrinology* **132** 1359–1366. (doi:10.1210/endo.132.3.8440191)
- Dai JC, He P, Chen X & Greenfield EM 2006 TNF α and PTH utilize distinct mechanisms to induce IL-6 and RANKL expression with markedly different kinetics. *Bone* **38** 509–520. (doi:10.1016/j.bone.2005.10.007)
- Dobnig H & Turner RT 1995 Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. *Endocrinology* **136** 3632–3638. (doi:10.1210/endo.136.8.7628403)
- Greenfield EM, Shaw SM, Gornik SA & Banks MA 1995 Adenyl cyclase and interleukin 6 are downstream effectors of parathyroid hormone resulting in stimulation of bone resorption. *Journal of Clinical Investigation* **96** 1238–1244. (doi:10.1172/JCI118157)
- Greenfield EM, Horowitz MC & Lavish SA 1996 Stimulation by parathyroid hormone of interleukin-6 and leukemia inhibitory factor expression in osteoblasts is an immediate-early gene response induced by cAMP signal transduction. *Journal of Biological Chemistry* **271** 10984–10989. (doi:10.1074/jbc.271.18.10984)
- Guo J, Liu M, Yang D, Bouxsein ML, Saito H, Galvin RJ, Kuhstoss SA, Thomas CC, Schipani E, Baron R *et al.* 2010 Suppression of Wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation. *Cell Metabolism* **11** 161–171. (doi:10.1016/j.cmet.2009.12.007)
- Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S-I, Nakajima K, Koyama K, Iwamatsu A *et al.* 1986 Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature* **324** 73–76. (doi:10.1038/324073a0)
- Hodsman AB, Bauer DC, Dempster DW, Dian L, Hanley DA, Harris ST, Kendler DL, McClung MR, Miller PD, Olszynski WP *et al.* 2005 Parathyroid hormone and teriparatide for the treatment of osteoporosis: a review of the evidence and suggested guidelines for its use. *Endocrine Reviews* **26** 688–703. (doi:10.1210/er.2004-0006)
- Horwood NJ, Kartsogiannis V, Quinn JM, Romas E, Martin TJ & Gillespie MT 1999 Activated T lymphocytes support osteoclast formation *in vitro*. *Biochemical and Biophysical Research Communications* **265** 144–150. (doi:10.1006/bbrc.1999.1623)
- Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM & Manolagas SC 1999 Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *Journal of Clinical Investigation* **104** 439–446. (doi:10.1172/JCI6610)
- Johnson RW, White JD, Walker EC, Martin TJ & Sims NA 2014a Myokines (muscle-derived cytokines and chemokines) including ciliary neurotrophic factor (CNTF) inhibit osteoblast differentiation. *Bone* **64C** 47–56. (doi:10.1016/j.bone.2014.03.053)
- Johnson RW, Brennan HJ, Vrahnas C, Poulton IJ, McGregor NE, Standal T, Walker EC, Koh TT, Nguyen H, Walsh NC *et al.* 2014b The primary function of gp130 signaling in osteoblasts is to maintain bone formation and strength, rather than promote osteoclast formation. *Journal of Bone and Mineral Research* **29** 1492–1505. (doi:10.1002/jbmr.2159)
- Kartsogiannis V, Moseley J, McKelvie B, Chou ST, Hards DK, Ng KW, Martin TJ & Zhou H 1997 Temporal expression of PTHrP during endochondral bone formation in mouse and intramembranous bone formation in an *in vivo* rabbit model. *Bone* **21** 385–392. (doi:10.1016/S8756-3282(97)00180-4)
- Keller H & Kneissel M 2005 SOST is a target gene for PTH in bone. *Bone* **37** 148–158. (doi:10.1016/j.bone.2005.03.018)
- Khosla S, Westendorf JJ & Oursler MJ 2008 Building bone to reverse osteoporosis and repair fractures. *Journal of Clinical Investigation* **118** 421–428. (doi:10.1172/JCI33612)
- Kim SW, Pajevic PD, Selig M, Barry KJ, Yang JY, Shin CS, Baek WY, Kim JE & Kronenberg HM 2012 Intermittent parathyroid hormone administration converts quiescent lining cells to active osteoblasts. *Journal of Bone and Mineral Research* **27** 2075–2084. (doi:10.1002/jbmr.1665)
- Kramer I, Loots GG, Studer A, Keller H & Kneissel M 2010 Parathyroid hormone (PTH)-induced bone gain is blunted in SOST overexpressing and deficient mice. *Journal of Bone and Mineral Research* **25** 178–189. (doi:10.1359/jbmr.090730)
- Kulkarni NH, Halladay DL, Miles RR, Gilbert LM, Frolik CA, Galvin RJ, Martin TJ, Gillespie MT & Onyia JE 2005 Effects of parathyroid hormone on Wnt signaling pathway in bone. *Journal of Cellular Biochemistry* **95** 1178–1190. (doi:10.1002/jcb.20506)
- Lee SK & Lorenzo JA 1999 Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. *Endocrinology* **140** 3552–3561. (doi:10.1210/endo.140.8.6887)
- Lindsay R, Zhou H, Cosman F, Nieves J, Dempster DW & Hodsman AB 2007 Effects of a one-month treatment with PTH(1–34) on bone formation on cancellous, endocortical, and periosteal surfaces of the human ilium. *Journal of Bone and Mineral Research* **22** 495–502. (doi:10.1359/jbmr.070104)
- Lu Y, Xie Y, Zhang S, Dusevich V, Bonewald LF & Feng JQ 2007 DMP1-targeted Cre expression in odontoblasts and osteocytes. *Journal of Dental Research* **86** 320–325. (doi:10.1177/154405910708600404)
- Ma YL, Cain RL, Halladay DL, Yang X, Zeng Q, Miles RR, Chandrasekhar S, Martin TJ & Onyia JE 2001 Catabolic effects of continuous human PTH (1–38) *in vivo* is associated with sustained stimulation of RANKL and inhibition of osteoprotegerin and gene-associated bone formation. *Endocrinology* **142** 4047–4054. (doi:10.1210/endo.142.9.8356)
- McGregor NE, Poulton IJ, Walker EC, Pompolo S, Quinn JM, Martin TJ & Sims NA 2010 Ciliary neurotrophic factor inhibits bone formation and plays a sex-specific role in bone growth and remodeling. *Calcified Tissue International* **86** 261–270. (doi:10.1007/s00223-010-9337-4)
- Miao D, He B, Jiang Y, Kobayashi T, Sorocanu MA, Zhao J, Su H, Tong X, Amizuka N, Gupta A *et al.* 2005 Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1–34. *Journal of Clinical Investigation* **115** 2402–2411. (doi:10.1172/JCI24918)
- Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A, Wagner EF *et al.* 2011 Evidence for

- osteocyte regulation of bone homeostasis through RANKL expression. *Nature Medicine* **17** 1231–1234. (doi:10.1038/nm.2452)
- Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK *et al.* 2001 Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *New England Journal of Medicine* **344** 1434–1441. (doi:10.1056/NEJM200105103441904)
- O'Brien CA, Gubrij I, Lin SC, Saylor RL & Manolagas SC 1999 STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF- κ B ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. *Journal of Biological Chemistry* **274** 19301–19308. (doi:10.1074/jbc.274.27.19301)
- Palmqvist P, Persson E, Conaway HH & Lerner UH 2002 IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF- κ B ligand, osteoprotegerin, and receptor activator of NF- κ B in mouse calvariae. *Journal of Immunology* **169** 3353–3362. (doi:10.4049/jimmunol.169.6.3353)
- Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM & Recker RR 1987 Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *Journal of Bone and Mineral Research* **2** 595–610. (doi:10.1002/jbmr.5650020617)
- Reeve J, Meunier PJ, Parsons JA, Bernat M, Bijvoet OL, Courpron P, Edouard C, Klenerman L, Neer RM, Renier JC *et al.* 1980 Anabolic effect of human parathyroid hormone fragment on trabecular bone in involutional osteoporosis: a multicentre trial. *BMJ* **280** 1340–1344. (doi:10.1136/bmj.280.6228.1340)
- Richards CD, Langdon C, Deschamps P, Pennica D & Shaughnessy SG 2000 Stimulation of osteoclast differentiation *in vitro* by mouse oncostatin M, leukaemia inhibitory factor, cardiotrophin-1 and interleukin 6: synergy with dexamethasone. *Cytokine* **12** 613–621. (doi:10.1006/cyto.1999.0635)
- Romas E, Udagawa N, Zhou H, Tamura T, Saito M, Taga T, Hilton DJ, Suda T, Ng KW & Martin TJ 1996 The role of gp130-mediated signals in osteoclast development: regulation of interleukin 11 production by osteoblasts and distribution of its receptor in bone marrow cultures. *Journal of Experimental Medicine* **183** 2581–2591. (doi:10.1084/jem.183.6.2581)
- Saini V, Marengi DA, Barry KJ, Fulzele KS, Heiden E, Liu X, Dedic C, Maeda A, Lotinun S, Baron R *et al.* 2013 Parathyroid hormone (PTH)/PTH-related peptide type 1 receptor (PPR) signaling in osteocytes regulates anabolic and catabolic skeletal responses to PTH. *Journal of Biological Chemistry* **288** 20122–20134. (doi:10.1074/jbc.M112.441360)
- Sims NA & Walsh NC 2010 GP130 cytokines and bone remodelling in health and disease. *BMB Reports* **43** 513–523. (doi:10.5483/BMBRep.2010.43.8.513)
- Sims NA, Brennan K, Spaliviero J, Handelsman DJ & Seibel MJ 2006 Perinatal testosterone surge is required for normal adult bone size but not for normal bone remodeling. *American Journal of Physiology. Endocrinology and Metabolism* **290** E456–E462. (doi:10.1152/ajpendo.00311.2005)
- Suva LJ, Winslow GA, Wettenhall RE, Hammonds RG, Moseley JM, Diefenbach-Jagger H, Rodda CP, Kemp BE, Rodriguez H, Chen EY *et al.* 1987 A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. *Science* **237** 893–896. (doi:10.1126/science.3616618)
- Takay FM, Tonna S, Ho PW, Crimée-irwin B, Baker EK, Martin TJ & Sims NA 2013 EphrinB2/EphB4 inhibition in the osteoblast lineage modifies the anabolic response to parathyroid hormone. *Journal of Bone and Mineral Research* **28** 912–925. (doi:10.1002/jbmr.1820)
- Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, Koishihara Y, Ohsugi Y, Kumaki K, Taga T *et al.* 1993 Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. *PNAS* **90** 11924–11928. (doi:10.1073/pnas.90.24.11924)
- Tonna S, Takay FM, Vrahnas C, Crimée-irwin B, Ho PW, Poulton IJ, Brennan HJ, McGregor NE, Allan EH & Nguyen H 2014 EphrinB2 signaling in osteoblasts promotes bone mineralization by preventing apoptosis. *FASEB Journal* [in press]. (doi:10.1096/fj.14-254300)
- Torreggiani E, Matthews BG, Pejda S, Matic I, Horowitz MC, Grcevic D & Kalajic I 2013 Preosteocytes/osteocytes have the potential to dedifferentiate becoming a source of osteoblasts. *PLoS ONE* **8** e75204. (doi:10.1371/journal.pone.0075204)
- Tosato G, Seamon KB, Goldman ND, Sehgal PB, May LT, Washington GC, Jones KD & Pike SE 1988 Monocyte-derived human B-cell growth factor identified as interferon-beta 2 (BSF-2, IL-6). *Science* **239** 502–504. (doi:10.1126/science.2829354)
- Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, Nakagawa N, Yasuda H, Goto M, Tsuda E *et al.* 1999 Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF- κ B ligand. *Bone* **25** 517–523. (doi:10.1016/S8756-3282(99)00210-0)
- Walker EC, McGregor NE, Poulton IJ, Pompolo S, Allan EH, Quinn JM, Gillespie MT, Martin TJ & Sims NA 2008 Cardiotrophin-1 is an osteoclast-derived stimulus of bone formation required for normal bone remodeling. *Journal of Bone and Mineral Research* **23** 2025–2032. (doi:10.1359/jbmr.080706)
- Walker EC, McGregor NE, Poulton IJ, Solano M, Pompolo S, Fernandes TJ, Constable MJ, Nicholson GC, Zhang JG, Nicola NA *et al.* 2010 Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. *Journal of Clinical Investigation* **120** 582–592. (doi:10.1172/JCI40568)
- Walker EC, Poulton IJ, McGregor NE, Ho PW, Allan EH, Quach JM, Martin TJ & Sims NA 2012 Sustained RANKL response to parathyroid hormone in oncostatin M receptor-deficient osteoblasts converts anabolic treatment to a catabolic effect *in vivo*. *Journal of Bone and Mineral Research* **27** 902–912. (doi:10.1002/jbmr.1506)
- Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC & O'Brien CA 2011 Matrix-embedded cells control osteoclast formation. *Nature Medicine* **17** 1235–1241. (doi:10.1038/nm.2448)
- Yao GQ, Wu JJ, Troiano N & Insogna K 2011 Targeted overexpression of Dkk1 in osteoblasts reduces bone mass but does not impair the anabolic response to intermittent PTH treatment in mice. *Journal of Bone and Mineral Metabolism* **29** 141–148. (doi:10.1007/s00774-010-0202-3)

Received in final form 19 August 2014

Accepted 15 September 2014

Accepted Preprint published online 16 September 2014