Loss of the nutrient sensor Tas1R3 leads to reduced bone resorption

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The TAS1R family of heterotrimeric G protein-coupled receptors participates in monitoring energy and nutrient needs. TAS1R3 is a bi-functional protein that either recognizes amino acids such as glycine and L-glutamate or sweet molecules such as sucrose and fructose when dimerized with TAS1R1 or TAS1R2, respectively. Loss of TAS1R3 expression leads to impaired mTORC1 signaling and increased autophagy, indicating that signaling through this receptor is critical for assessing nutrient needs. Recently, it was reported that global deletion of TAS1R3 expression in mice (Tas1R3 mutant) leads to increased cortical bone mass and trabecular remodeling (Simon et al 2014) but the underlying cellular mechanism leading to this phenotype remains unclear. To address this open question, we quantified bone turnover markers in serum from 20-week-old wild type and Tas1R3 mutant mice and found that levels of the resorption marker Collagen Type I C-telopeptide (CTx) were reduced on average by >60% in the absence of TAS1R3 expression. Levels of the bone formation marker Procollagen Type I N-terminal Propeptide (P1NP) tend to be higher in Tas1R3 mutant mice but this finding did not reach statistical significance. These preliminary results suggest that high bone mass in Tas1R3 mutant mice is due to uncoupled bone remodeling with reduced osteoclast function. We examined the skeletal expression profile of Tas1R3 in order to determine the cellular compartment(s) in which TAS1R3 impacts bone remodeling. Consistent with the observed defect in bone resorption in Tas1R3 mutant mice, Tas1R3 mRNA is expressed in primary osteoclasts obtained from wild type mice. However, Tas1R3 mRNA is also present in marrow-free humerii, primary bone marrow stromal cells, and several osteoblast-like cell lines, raising the possibility that osteoblast-to-osteoclast communication may be disrupted in Tas1R3 mutant mice. Collectively, these findings provide the rationale for future experiments examining the cell type-dependent role for TAS1R3 function in nutrient sensing in postnatal bone remodeling and differentiation of osteoclasts and osteoblasts.

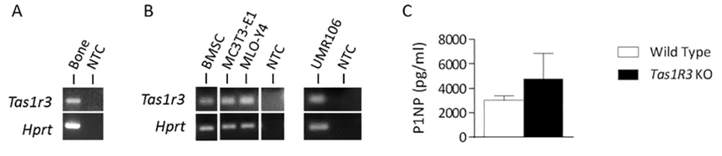

Fig. 1 Expression of *Tas1r3* in bone and bone cells and examination of bone formation in *Tas1R3* knock-out mice. A, B: RT-PCR for *Tas1r3* expression in cDNA from marrow-free bone (A) and bone-related cell types (B). *Hprt* serves as housekeeping control. NTC: no DNA template control. Vertical bars represent removal of intervening lane(s). **C:** Examination of bone formation via levels of Procollagen Type I N-terminal Propeptide (P1NP) in serum from 20-week-old wild type and *Tas1R3* knock-out (KO) mice: wild type: 981±407.5 pg/ml, n=3; Tas1R3 mutant 2009±421.5 pg/ml, n=3; p<0.15 by unpaired t test.

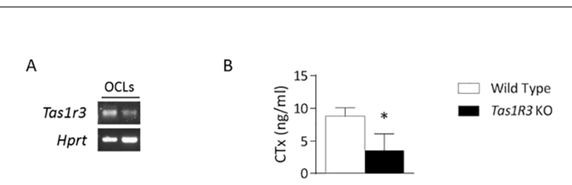

Fig. 2 Expression of *Tas1r3* in differentiated osteoclasts and examination of bone resorption in *Tas1R3* knock-out mice. A: RT-PCR for *Tas1r3* expression in cDNA from murine non-adherent bone marrow cells treated with M-CSF and RANKL (OCLs). *Hprt* serves as housekeeping control. Results from two biological replicates are shown. **B:** Examination of bone resorption via levels of Collagen Type I C-telopeptide (CTx) in serum from 20-week-old wild type and *Tas1R3* knock-out (KO) mice: wild type: 8.791±0.75 ng/ml, n=3; Tas1R3 mutant 3.248±1.14 ng/ml, n=4; p<0.02 by unpaired t test.

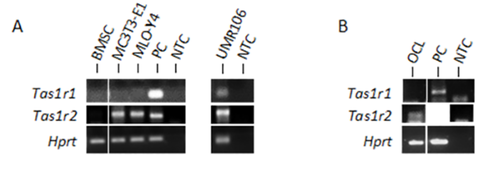

Fig. 3 Expression of *Tas1r1* and *Tas1r2* in bone-related cells. RT-PCR for *Tas1r1* and *Tas1R2* expression in cDNA from the sources as indicated. PC: positive control using C2C12 cDNA. NTC: no DNA template control.

Preliminary Implications:

- Previous work by Simon et al (2014) implicated Tas1R3 in postnatal bone remodeling.
- Our results demonstrate broad expression of Tas1R family members in the skeleton and in bone-related cells: undifferentiated murine bone marrow stromal cells express Tas1R3 alone; MC3T3-E1 cells and MLO-Y4 cells express Tas1R2 and Tas1R3; UMR-106 cells express Tas1R1, Tas1R2, and Tas1R3; differentiated murine osteoclasts express Tas1R2 and Tas1R3.
- Global *Tas1R3* deficiency in mice leads to decreased bone resorption and a tendency toward higher bone formation.

Future Directions:

- Evaluation of osteoblast and osteoclast numbers, bone formation rate, and bone resorption rate via static and dynamic histomorphometry.
- Determination of TAS1R3 function in differentiation of osteoblasts and osteoclasts using in vitro differentiation studies.
- Examination of global versus skeletal-specific role for TAS1R3 via specific deletion of *Tas1R3* in skeletal cells using conditional KO strategy.
- Generation of *Tas1R1/Tas1R3* and *Tas1R2/Tas1R3* double knockout mice to implicate which Tas1R ligands are involved in postnatal bone remodeling.
- Genome-wide association study to examine the correlation between SNPs in Tas1R family members and human bone mass.

Comments and Questions are welcome:

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