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IL15RA is required for osteoblast function and bone mineralization

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ABSTRACT

Interleukin-15 receptor alpha (IL15RA) is an important component of interleukin-15 (IL15) pro-inflammatory signaling. In addition, IL15 and IL15RA are present in the circulation and are detected in a variety of tissues where they influence physiological functions such as muscle contractility and overall metabolism. In the skeletal system, IL15RA was previously shown to be important for osteoclastogenesis. Little is known, however, about its role in osteoblast function and bone mineralization. In this study, we evaluated bone structural and mechanical properties of an *Il15ra* whole-body knockout mouse (Il15ra$^{-/-}$) and used *in vitro* and bioinformatic analyses to understand the role IL15/IL15RA signaling on osteoblast function. We show that lack of IL15RA decreased bone mineralization *in vivo* and in isolated primary osteogenic cultures, suggesting a cell-autonomous effect. Il15ra$^{-/-}$ osteogenic cultures also had reduced *Rankl*/*Opg* mRNA ratio, indicating defective osteoblast/osteoclast coupling. We analyzed the transcriptome of primary pre-osteoblasts from normal and Il15ra$^{-/-}$ mice and identified 1150 genes that were differentially expressed at a FDR of 5%. Of these, 844 transcripts were upregulated and 306 were downregulated in Il15ra$^{-/-}$ cells. The largest functional clusters, highlighted using DAVID analysis, were related to metabolism, immune response, bone mineralization and morphogenesis. The transcriptome analysis was validated by qPCR of some of the most significant hits. Using bioinformatic approaches, we identified candidate genes, including *Cd200* and *Enpp1*, that could contribute to the reduced mineralization. Silencing *Il15ra* using shRNA in the calvarial osteoblast MC3T3-E1 cell line decreased ENPP1 activity. Taken together, these data support that IL15RA plays a cell-autonomous role in osteoblast function and bone mineralization.

1. Introduction

Maintenance of bone mass requires a dynamic equilibrium between bone formation by osteoblasts and resorption by osteoclasts. Perturbation of this balance leads to conditions such as osteoporosis (decreased bone mass) or osteopetrosis (increased bone mass). Within the bone marrow, osteoblasts originate from mesenchymal progenitor cells and are responsible for deposition of newly formed bone matrix [1]. Osteoclasts, derived from the monocyte/macrophage hematopoietic lineage, adhere to and degrade bone matrix, a key function in the bone remodeling process that is needed to establish and maintain healthy bone quality [2]. Coupling between these two cell types occurs through RANKL (receptor activator of NF-kB ligand)-RANK-OPG (osteoprotegerin) signaling. The cytokine RANKL is secreted by activated osteoblasts, and by B and T-cells, and stimulates osteoclast differentiation upon binding to its receptor RANK expressed on the osteoclast's surface. OPG acts as a decoy receptor for RANKL, therefore antagonizing RANKL binding to RANK [3]. Bone resorption by osteoclasts releases factors such as semaphorin-4D, TGFβ1, and IGF1 from the bone matrix, which in turn regulate recruitment of mesenchymal progenitor cells and osteoblast differentiation [4]. In addition to RANKL, several other cytokines such as tumor necrosis factor α (TNFα) and interleukins (IL1, IL6, IL7, IL15, IL17) regulate bone formation and maintenance and have been implicated in bone and cartilage pathologies including osteoporosis and rheumatoid arthritis [5–7].

IL15 is a widely expressed pro-inflammatory cytokine and also functions as a myokine that is secreted from muscle in response to exercise [8–12]. Elevated IL15 receptor alpha (IL15RA) levels are found in the

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synovial fluid of patients affected by rheumatoid arthritis and other chronic inflammatory diseases that are associated with bone loss [7,13]. IL15 was shown to participate in both activation and turnover of osteoclasts mediated by NK cells [14,15]. Absence of IL15 signaling impairs osteoclast activity and protects against trabecular bone loss in ovariectomized mice [16]. Single nucleotide polymorphisms (SNPs) of the *IL15RA* gene have been associated with bone volume [17] as well as susceptibility to ossification of the posterior longitudinal ligament of the spine [18]. While the effects of IL15/IL15RA signaling on osteoclast function are known, the understanding of the effects of these signals on osteoblast function and on the coupling between osteoblasts and osteoclasts remains limited.

Previously, we and others have demonstrated that ablation of *Il15ra* increases spontaneous locomotor activity [19,20], improves fatigue resistance and reprograms energy metabolism favoring the maintenance of a lean phenotype [21]. Given the established crosstalk between exercise, metabolism and the musculoskeletal system [22,23], we examined the effects of IL15/IL15RA signaling on bone mineralization and osteoblast function in a mouse model lacking *Il15ra* (Il15ra$^{-/-}$). Using *ex vivo* bone imaging, mechanical testing and histological analysis, we evaluated bone mineralization and morphogenesis in Il15ra$^{-/-}$ femurs. Primary cultures of mouse osteoblast precursors from Il15ra$^{-/-}$ and control bones were used to test for a cell autonomous role of IL15RA in osteoblast function. Further, we combined bioinformatics approaches and transcriptome analysis to identify the mechanisms connecting IL15/IL15RA signaling to bone mineralization and phosphate homeostasis.

2. Methods

2.1. Animals

All animal experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania. 11 weeks old (unless differently stated) male Il15ra$^{-/-}$ (stock no. 003723) and background control B6129SF2/J (stock no. 101045) mice were purchased from Jackson Laboratories and housed at 22 °C under a 12-hour light/12-hour dark cycle with food and water provided *ad libitum*. For double labeling, mice were injected intraperitoneally with 20 mg/kg Calcein 6 days before being sacrificed, and with 200 mg/kg Xylenol orange 1 day before being sacrificed.

2.1.1. Bone micro-computed tomography (μCT) and three-point bending

μCT (microCT 35, ScancoMedical AG, Brüttisellen, Switzerland) was used to determine structural parameters of femur trabecular (1.2 mm region proximal to growth plate, 6 μm isotropic voxels) and cortical regions (0.3 mm at 50% length, 6 μm isotropic voxels), as well as tibia cortical region (1.2 mm region distal to growth plate, 6 μm isotropic voxels). Trabecular bone mineral density (BMD), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), trabecular number (Tb.N), structure model index (SMI), connectivity density (Tb.Conn.D), degree of anisotropy (DA), bone surface (BS), cortical tissue mineral density (Ct.TMD), cortical area (Ct.Ar) and cortical bone thickness (Ct.Th) were calculated by 3D standard microstructural analysis.

For 3-point bending test, femurs were placed in a consistent orientation and continuously hydrated in phosphate-buffered saline prior to test initiation. The span between the lower supports was set to 10 mm. The upper contact point was aligned at the mid-point of the lower supports. The upper actuator was ramped at a rate of 0.3 mm/s till failure of the specimen was achieved. μCT computed tomography scans of the mid-diaphyseal region were utilized to calculate the second moment of inertia of the samples. Flexural rigidity (N/mm^2), was calculated together with load at failure (N), displacement at failure (mm), and second moment of area (mm^4).

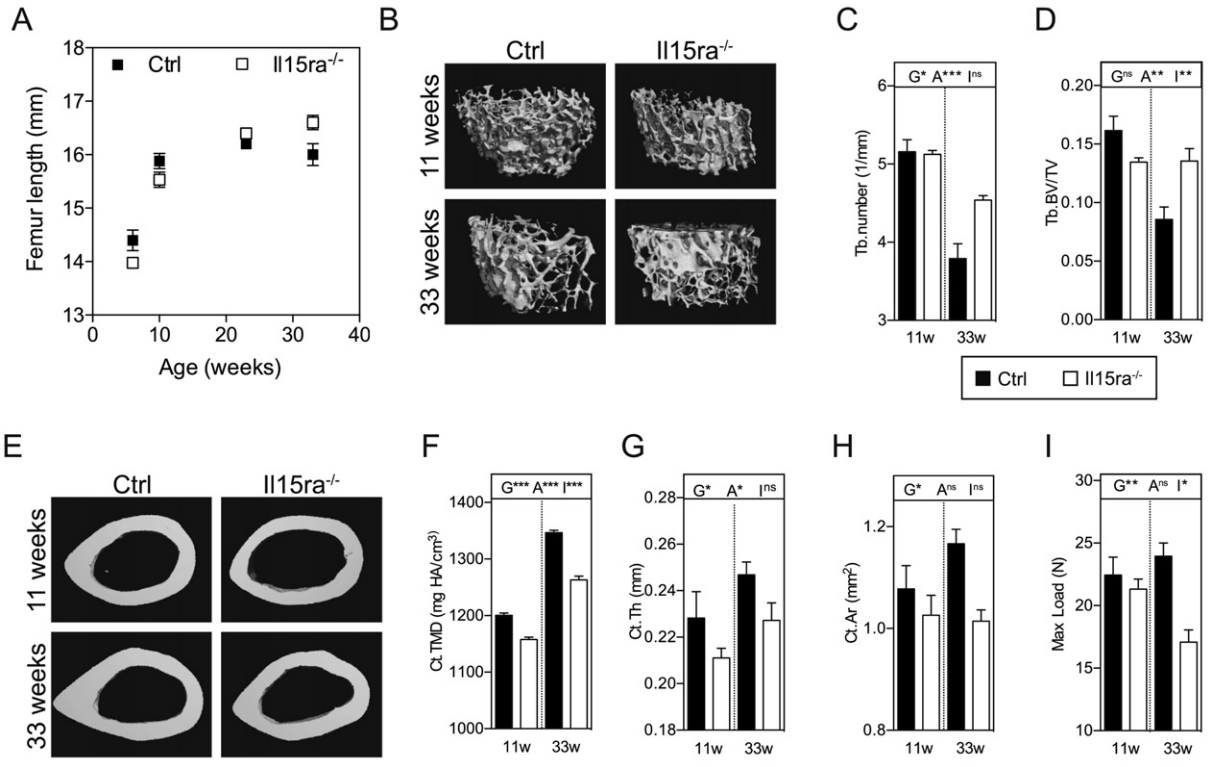

Fig. 1. Absence of IL15RA preserves trabecular bone structure but decreases cortical bone quality. (A) Femur length at different ages (n = 4–6/timepoint); (B) representative rendered microCT scans of femur trabeculae from control and Il15ra$^{-/-}$ mice at 11 and 33 weeks of age; trabeculae number (C) and trabecular bone volume/total volume (Tb.BV/TV) ratio (D) (n = 5). (E) Representative rendered microCT scans of femur cortical bone; cortical tissue mineral density (F), thickness (G) and area (H) (n = 5). (I) Maximal load as calculated with 3-points bending analysis (n = 5). G: effect of genotype; A: effect of age; I: interaction between genotype and age.