HES1 Disrupts the Transcriptome and Osteoclast Maturation in Male Mice Contributing to the Sexual Dimorphism in Bone Remodeling

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Disclosures: None

INTRODUCTION: Osteoclast differentiation is sexually dimorphic and cells of the myeloid lineage from female mice differentiate earlier and to a greater extent than cells from male mice. Accordingly, osteoclast number and bone resorption are increased in female mice compared to male mice, possibly contributing to the decrease in cancellous bone of female mice as they mature and age. However, the mechanisms responsible are poorly understood, and the differences cannot be explained solely on hormonal effects. Hairy Enhancer of Split (HES1) is a Notch target gene and transcriptional inhibitor that regulates the maturation of bone marrow-derived macrophages (BMM) into osteoclasts. As such, it may influence the sexual dimorphism of osteoclastogenesis and account, to an extent, for the sex differences in cancellous bone remodeling. To explore this possibility as well as novel mechanisms responsible for the sex-dependent differences in osteoclast maturation, bone resorption and remodeling, we tested the effects of HES1 in BMMs from male and female mice.

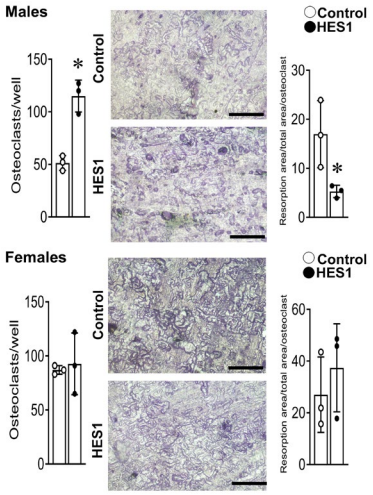
METHODS: The effects of HES1 on osteoclastogenesis were explored following its expression *in vivo* in undifferentiated cells of the myeloid lineage (*Lyz2* or *LysM*+ cells) from male and female mice using an inducible model of HES1 activation. To this end, *HES1* coding sequences were cloned into the *Rosa26* locus downstream of a *loxP* flanked STOP cassette, so that HES1 is expressed from the *Rosa26* locus following the deletion of the STOP cassette by Cre recombination. This was achieved by crossing *Rosa26-Hes1* mice with *Lyz2-Cre* mice. BMMs from *Lyz2-Cre;Rosa26-Hes1* (n=4) and control (n=4) mice were obtained and cultured in the presence of macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL) and analyzed for transcriptome profiles at the bulk and single cell (sc) resolution. The analysis was complemented by functional studies to determine the effect of HES1 on osteoclast differentiation and function in BMMs from male and female mice. Studies were approved by the Animal Care and Use Committee of UConn Health.

RESULTS: RNA-Seq of BMMs cultured in the presence of M-CSF and RANKL demonstrated 90 differentially expressed genes (DEG, log2C1, p adjusted < 0.05) in HES1-expressing osteoclasts from male mice, but only 4 DEGs in cells from female mice. Genes associated with RNA and metabolic processes were enriched in osteoclasts from male mice. Analysis of scRNA-Seq normalized data of pooled control and *Lyz2-Cre*$^{+}$*;Rosa26-Hes1* BMMs treated with M-CSF or M-CSF and RANKL established the presence of 10 - 12 well-defined cell clusters. Pseudotime trajectory analysis indicated a trajectory of clusters expressing genes associated with cell progenitors, hematopoietic stem cells/multipluripotent (HSC/MPP) undifferentiated and mature osteoclast cells. HES1 enhanced the number of osteoclast progenitors but did not influence the pseudotime trajectory in BMMs from female mice. In contrast, the progression of progenitor cells to mature osteoclasts was disrupted by HES1 in cells from male mice, a disruption reproduced by the removal of a defined cluster related to progenitors and mature cells. To assess the functional consequences of the HES1 expression in the myeloid lineage, BMMs were cultured in the presence of M-CSF and RANKL on bone slices. Cells from control female mice had a greater number of osteoclasts and bone resorptive capacity than cells from male mice and, in accordance with RNASeq results neither cell number nor bone resorption were affected by HES1 in cells from female mice (Figure lower panel). In contrast, osteoclasts were increased in number by HES1 in cells from male mice, but the number and area of resorption pits was decreased demonstrating that their terminal differentiation and resorptive capacity were impaired by HES1 (Figure upper panel). These results are in agreement with the disruption of osteoclast maturation by HES1 found in scRNASeq of BMMs from male mice.

DISCUSSION: BMMs from female mice mature as osteoclasts earlier and at a greater rate than cells from male mice, but neither their function nor maturation is influenced by HES1. In contrast, HES1 precludes the differentiation of progenitors to mature functional osteoclasts in cells from male mice. Increased activity of HES1 may explain the lower osteoclast number and bone resorption in male than in female mice as they mature and age.

SIGNIFICANCE: The work is significant because it addresses mechanisms responsible for osteoclast maturation and function that may explain the sexual dimorphism of osteoclast differentiation and function at a single cell resolution. Since Notch activity in skeletal cells increases with age, it is plausible to believe that the activity of its target gene HES1 increases as mice mature; HES1 would preclude osteoclast maturation and function in male but not in female mice explaining the sexually dimorphic alterations in bone resorption, remodeling and bone mass.

ACKNOWLEDGMENTS: Supported by NIAMS AR078149



Bone resorption is impaired in male (upper panel) but not in female BMMs (lower panel) by HES1.