Aldosterone inhibits osteoblast function through the mineralocorticoid receptor (MR)

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INTRODUCTION: Aldosterone signaling via the mineralocorticoid receptor (MR) is best known for effects in the cardiovascular and renal systems, promoting physiological changes like fluid retention and increased blood pressure. However, in addition, increased levels of aldosterone have also been shown to induce harmful changes in muscle and fat such as promoting myocyte apoptosis and increased insulin resistance which contributes to metabolic syndrome[1-2]. While the direct effects of aldosterone on bone have not been thoroughly investigated, high aldosterone levels, such as those that occur in primary aldosteronism, can serve as a risk factor for osteoporosis[3]. In earlier studies, our lab discovered (Figure 1A-B) that expression of the mineralocorticoid receptor (MR) increases with age in both mouse and human mesenchymal stem cells and in mouse bone tissue. We hypothesize that this increased MR expression facilitates increased aldosterone signaling through the MR, contributing to osteoblastic dysfunction that occurs with age. In the current study, we investigated the direct effects of aldosterone on osteoblastic function to determine whether it can regulate osteoblastic bone formation.

METHODS: Long bones from 6 and 21-month C57/BL6J female mice were collected at sacrifice. Bone marrow stromal cells (BMSCs) were isolated from the femur and tibia and cultured for 21 days in osteogenic medium (alpha MEM+20% FBS+1% antibiotic/antimycotic+50 µg/ml ascorbic acid + 10 mM beta glycerophosphate) and increasing amounts of aldosterone (MCE, Cat # HY113313) to assess mineralized matrix formation. Cultures were fixed in formalin and mineralized matrix was visualized by staining with alizarin red which was quantified using BioQuant software. Additional osteoblastic cultures were seeded onto glass slides coated with type 1 collagen, and RNAscope (ACD Bio) was used to observe mRNA expression of the MR, glucocorticoid receptor (GR) and type 1 collagen (Col1a1) at day 7 and day 21 of osteoblastic differentiation. To determine whether osteoblasts express a functional MR protein capable of regulating transcriptional activity, we transfected MC3T3 cells with an MMTV-Luc reporter plasmid featuring glucocorticoid response elements (GREs) in the DNA capable of binding MR. The MMTV-transfected cells were treated for 24 hours with either aldosterone or dexamethasone (a glucocorticoid, used as a positive control for MMTV-Luc activation) and then collected cell lysates to measure firefly luciferase activity normalized to Renilla luciferase (Promega Dual Luciferase Reporter Assay, Cat # E1910 and Agilent Biotek Cytation 5 instrument). Datasets with two experimental groups were statistically compared with Student's t-tests, and datasets with more than two groups were statistically compared via ANOVA. All animal experiments were performed with approval from that Augusta University IACUC.

RESULTS: We previously showed that MR expression increases with age in both mouse (p=0.011) and human osteoblast progenitor cells (p=0.048) (Figure 1A-B). In the current study, we verified this finding in osteoblasts, showing that Col1a1 expressing cells from aged mice have higher MR expression than cells from younger mice, especially at day 21 of differentiation (Figure 1C). In contrast, expression of GR tended to be lower in the aged cells, especially early (Day 7) in the time course of osteoblastic differentiation (Figure 1C). To test the functionality of MR in a pre-osteoblastic cell line and to determine whether it could be activated by aldosterone, we utilized the well-established MMTV-Luc plasmid, which features GRE binding sites capable of binding both GR and MR. While aldosterone is capable of binding to GR, it does so with much lower affinity than to MR and is a very weak activator of GR (EC_{50} > 1000 nM [4-5]). MC3T3 cells treated with 100nM aldosterone showed activation of the MMTV-Luc reporter as compared to vehicle treated cells (p=0.042), similar to the activation of this reporter by the glucocorticoid dexamethasone (Figure 1D). BMSC-derived osteoblasts derived from both young and mold mice, treated with 100nM of aldosterone in culture tended (p=0.067) to show decreased mineralized matrix production as compared to vehicle-treated cells (Figure 1E-F).

DISCUSSION: These data suggest that both primary and immortalized osteoblasts express functional MR and that aldosterone has a direct and negative effect on osteoblast function. Interestingly, although we confirmed our earlier findings suggesting increased expression of MR in bone with age, this trend seems most apparent at later stages of osteoblastic culture, concurrent with robust expression of mRNA for type 1 collagen. In contrast, expression of the GR, which we previously reported to be critical for osteoblastic function[6], seems to be highest at early stages of osteoblastic differentiation (day 7) and to decrease in magnitude with age. A limitation of this study is that cultures were solely derived from female mice, although comparable experiments in males are currently ongoing. In addition, as there currently exists no antibody capable of reliably recognizing murine MR protein, our studies were necessarily confined to gene expression and reporter assay endpoints. We recognize that aldosterone concentrations employed in this study are supraphysiological (with physiological concentrations of aldosterone on the order of 0.1nM in healthy patients to >1 nM in patients with primary aldosteronism), however, we note that renin to aldosterone ratio plays a major role in signaling and that aldosterone concentrations of 100 to 500 nM are commonly utilized for in vitro studies[2] which are capable of faithfully mimicking MR-mediated signaling changes known to occur in vivo.

SIGNIFICANCE/CLINICAL RELEVANCE: These results suggest that aldosterone has a direct and negative impact on osteoblast function, confirming the existence of functional MR-mediated signaling in osteoblasts. As our results suggest that MR expression increases in both mouse and human osteoblast-lineage cells with age, this suggests that aldosterone and its activation of MR may contribute to age-related osteoblastic dysfunction and osteoporosis.

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Figure Legend: Figure 1: (A) Human CD271+ MSC, Young: <40 yrs, Old: >70 yrs (B) Wildtype C57BL/6 mice sorted mesenchymal stem cells (MSC) (C) MMTV-Luc reporter plasmid shows activation by 100nM aldosterone, or dexamethasone as compared to vehicle (EtOH) (D-E) Mineralized matrix production tends to be blunted by aldosterone Representative RNAscope images from days 7 and 21 of 6 and 21 month BMSCs treated with osteogenic media showing mRNA for MR, GR and Col1a1.