

Alendronate Alters Cartilage Transcriptome in Early-Stage Load-Induced Post-Traumatic Osteoarthritis

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Introduction: Subchondral bone remodeling is altered in osteoarthritis (OA) progression. During early-stage disease bone remodeling is elevated, and bone loss occurs, whereas in late-stage disease bone remodeling is decreased, leading to increased bone formation¹. Immediate suppression of bone remodeling may be an effective therapy for post-traumatic OA (PTOA). Consequently, antiresorptive bisphosphonates have been studied as potential disease-modifying drugs² with mixed results in clinical trials of OA³. Alendronate (ALN) is an FDA-approved bisphosphonate for osteoporosis. In a mouse model of load-induced PTOA, immediate ALN treatment attenuated cartilage damage at 3- and 6-weeks after a single bout of loading⁴. To gain insight into the mechanism by which ALN treatment led to reduced cartilage damage in load-induced PTOA, we determined the early-stage cartilage transcriptomic changes in the saline (VEH) and ALN-treated mice after a single bout of cyclic loading. We hypothesized that the tissue-level structural changes previously seen in ALN treated mice 3- and 6-weeks after a single bout of cyclic tibial loading would be identifiable in transcriptomic alterations 1-week post PTOA initiation³.

Methods: Under IACUC approval, the left hindlimbs of 26-week-old male C57Bl/6J mice underwent a single bout of cyclic tibial loading (9N peak load, 1200 cycles, 4Hz)⁵. The right hindlimbs were nonloaded controls. Mice were randomized into two treatment groups, and were injected with saline (VEH, IP, n=8) or ALN (73µg/kg/day, IP, n=8) immediately following loading for 5 consecutive days. Mice were euthanized 1-week after loading, and tibial cartilage was microdissected and stabilized in RNAlater for RNA isolation⁶. 3' RNA sequencing was performed at the Cornell Genomics Core. STAR was used to align transcriptomes. We compared loaded and control limbs for differentially expressed genes (DEGs, EdgeR, FDR<0.05), and then the DEGs were used to determine the top enriched biological pathways (GSEA, FDR<0.05)⁷.

Results: Using RNA-seq, we identified 775 DEGs comparing loaded vs contralateral control limbs in the VEH-treated group, and 757 DEGs in the ALN-treated group, with 469 overlapping DEGs between treatments (Fig. 1). Only one common DEG, *Tmc8*, was downregulated in VEH-treated loaded limbs and upregulated in ALN-loaded limbs compared to control limbs. These DEGs included well known OA associated genes. *Plod2*, *Trps1*, *Gdf5*, *Il11*, *Piezo2*, *Igf1*, *Postn*, and *S100a4* were differentially expressed with both treatments (Fig 2). *Comp*, *Ogn*, *Trpv4*, *Nfatc2*, *Wnt5a*, *Ngf*, and *Sox11* were differentially expressed only in the loaded VEH group, and *Col10a1*, *Fgfr3*, *Jun*, and *Dact1* were differentially expressed only in the ALN-treated cartilage. Pathway analyses identified treatment-dependent differences. Cartilage development, cartilage differentiation, appendage development and connective tissue development were only enriched in the VEH group, whereas osteoblast regulatory pathways, muscle development, and Wnt signaling pathways were only differentially enriched in the ALN-treated mice (Fig. 3).

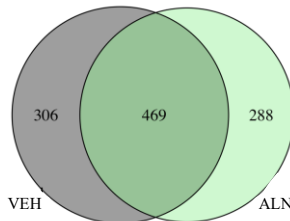


Figure 1: DEGs in loaded vs control limbs in VEH and ALN

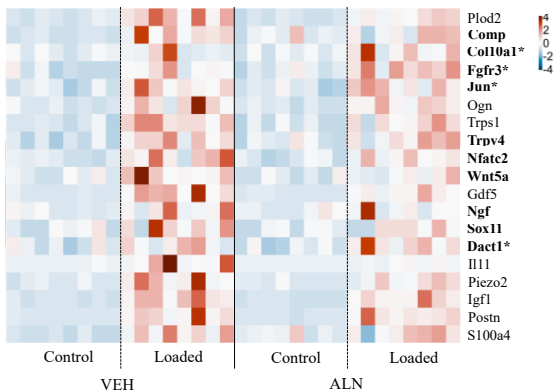


Figure 2: Normalized counts for DEGs of interest. Both VEH and ALN, **VEH only**, **ALN only***

Discussion: RNA-seq analyses of tibial cartilage isolated at 1-week after a single bout of loading identified unique and overlapping DEGs and pathways comparing VEH- and ALN-treated mice. These transcriptomic differences could provide insight into the mechanism by which immediate ALN treatment attenuates cartilage damage. Enrichment of negative regulators of osteoblast differentiation in the ALN group suggests that ALN treatment impaired chondrocyte hypertrophy and osteoblast differentiation⁸, processes associated with cartilage damage in OA, and could explain the attenuated structural damage that we previously reported 3- and 6-weeks post loading³. Future work should further interrogate the exact mechanisms whereby ALN attenuates the progression of PTOA, and the impact of ALN treatment in the crosstalk between different joint compartments.

Significance: We identified transcriptomic changes that may attenuate PTOA development in response to antiresorptive treatment, highlighting the potential for antiresorptive drugs as PTOA therapeutics.

References: ¹Yuan+ 2014 ²Fernández-Martín+ 2021 ³Iwamoto+ 2009 ⁴Ziemian+ 2021 ⁵Ko+ 2013 ⁶Culley+ 2021 ⁷Antoinette+ 2022 ⁸Rutkovskiy+ 2016

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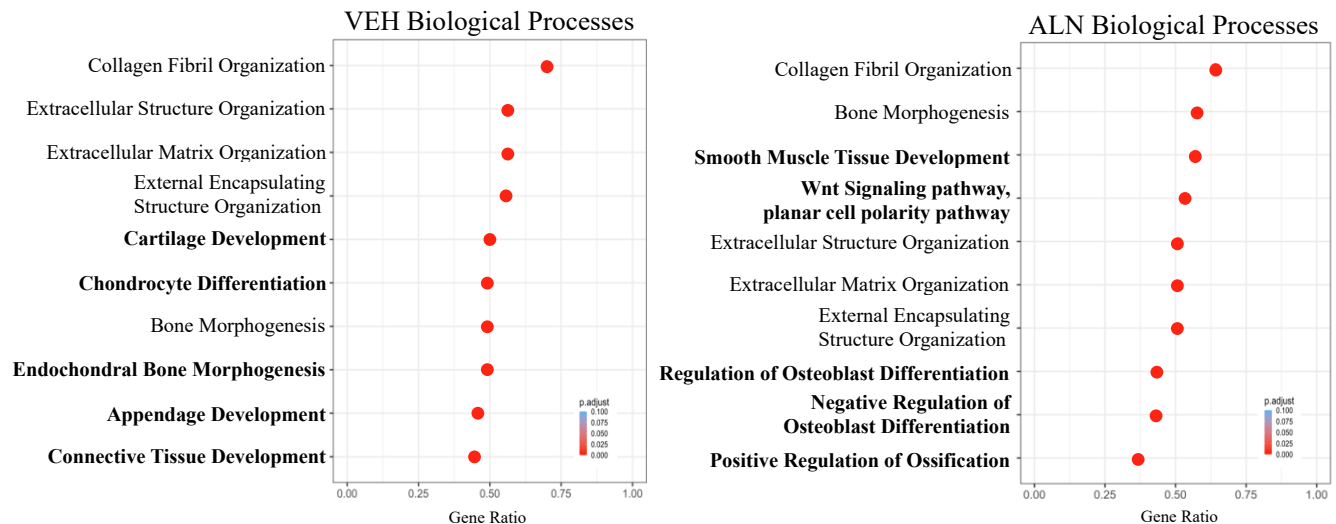


Figure 3: Top 10 upregulated biological processes in VEH and ALN groups. **Bold** = differentially regulated pathway