

## Osteoblast Transcriptome Changes During Iron Deficiency

Shyam Polaconda<sup>1</sup>, Gang Peng<sup>2</sup>, Erica Clinkenbeard<sup>2</sup>

<sup>1</sup>Indiana University School of Medicine; <sup>2</sup>Indiana University School of Medicine, Department of Medical and Molecular Genetics

### Introduction:

Chronic kidney disease (CKD) often results in iron deficiency anemia and bone loss. Previously, iron deficiency was found to impede normal osteoblast differentiation. To investigate regulatory mechanisms elicited by iron deficiency we utilized unbiased RNA-sequencing to evaluate transcriptome-level changes over the course of differentiation.

### Methods:

Mouse progenitor clone 2 (MPC-2) cells were differentiated in osteogenic media with or without an iron chelator, deferoxamine (DFO; 5  $\mu$ M). RNA was extracted at 3, 7, and 14 days after initiating differentiation. Library generation and sequence alignment was performed by the Center for Medical Genomics (CMG). Count-based algorithms implemented in R packages (edgeR and limma-voom; default parameters) identified differentially expressed genes between cells in the control and DFO treatment.

### Results:

RNA sequencing illustrated transcriptome changes due to iron deficiency including 63 upregulated/81 downregulated genes at day 3, 371 upregulated/780 downregulated genes at day 7, and 342 upregulated/739 downregulated genes at day 14. Importantly, day 14 controls clustered as transcriptional distinct whereas day 14 DFO treated cells clustered with early time points. As expected, genes involved in bone mineralization were suppressed with DFO including *Dmp1* (logFC: -5.92, p= 5.7e-10) and *Phex* (logFC: -4.17, p= 5.5e-16). Interestingly, genes such as *Ces2e*, *Gdf15*, and *Ano3*, not previously found associated with osteoblasts, were significantly upregulated after 3 days with DFO (logFC: 5.14, 2.7, and 2.5 respectively).

### Conclusions:

Transcriptomic changes occur even within 3 days of exposure to iron deficiency. Next steps include performing functional enrichment analysis, and validating expression level changes *in vitro* as well as within *in vivo* models of CKD-mediated bone loss.

### Scientific Impact and implications:

Iron deficiency is intricately linked to impaired osteoblast function. Understanding the underlying molecular mechanisms could elucidate novel avenues for anabolic bone therapies in CKD.