

Periosteal Cells are a Local Source of IGF-1 in bone

Madhura P Nijssure¹, Clifford J Rosen¹

¹MaineHealth Institute for Research

Disclosures: Madhura P. Nijssure (N), Clifford J. Rosen (5, 7a)

Introduction: The insulin-like growth factor (IGF) system, composed of IGFs, the IGF receptor (IGF1R), and IGF-binding proteins (IGFBP-1 to -6), plays a central role in skeletal development and bone remodeling. The liver is the primary source of circulating IGF-1; however, IGF-1 is also produced locally in bone and muscle, where it acts in a paracrine manner to stimulate bone formation and muscle growth². A reduction in circulating IGF-1 is a hallmark of calorie restriction (CR), an intervention widely adopted for weight loss, metabolic health, and longevity³. While CR is generally beneficial, it reduces circulating IGF-1 and accelerates bone and muscle loss. Our central hypothesis is that sustaining IGF-1 levels during CR can prevent skeletal deterioration. A key step toward this goal is defining the local cellular source of IGF-1 in bone and muscle, as well as the cell types capable of responding to IGF-1 via IGF1-R. Here, we analyzed a previously published single-cell RNA sequencing (scRNA-seq) dataset with CellChat and performed RNAScope to identify *Igf1* expressing populations within bone and muscle and explore their role in local IGF-1 mediated signaling.

Materials and Methods: We analyzed publicly available scRNA-seq data from young (8-10 weeks) and old (10 months) mice (both males and females), originally published by Liu et al. (GEO accession: GSE249528)⁴. We downloaded the processed count matrices for the periosteal stromal cells (PSC) from GEO, which included periosteal cells, as well as cells from the bone marrow. Cells expressing fewer than 300 or more than 7000 genes, or with >10% mitochondrial reads were excluded. We analyzed a total of 7321 cells. Downstream analyses including quality control, normalization, clustering and visualization were conducted using Seurat (v4). Dimensionality reduction was performed with PCA, which indicated 15 principal components that were used for downstream clustering. Graph-based clustering was run with a resolution parameter of 0.2. The resulting clusters were visualized with uniform manifold approximation and projection (UMAP). Clusters were annotated by applying periosteal cell markers identified using established periosteal, stromal, immune and endothelial markers. Intercellular communication networks were inferred using the CellChat R package (v 1.6.1). Ligand-receptor interactions were predicted using the built-in mouse reference database. Communication probability was calculated based on gene expression levels and normalized by the number of cells in each cluster. IGF signaling networks were visualized with bubble plots using netVisual_aggregate. Finally, to confirm our findings from the scRNA-seq data, we probed for *Igf1* by RNAScope, according to a previously published protocol⁵ in femurs from 16-week-old wildtype male mice from a C57BL/6J background.

Results: Our analysis showed 12 distinct clusters within this dataset (**Fig. 1 A**). We annotated fibrous layer periosteal cells with *Dpt* and *Cd34*, and cambium layer periosteal cells with *Postn*, and *Sp7*. The remaining clusters were annotated based on lineage-specific markers. Macrophages were identified by *Lyz2* and *Cd68*, immune cells by *Ptprc* and *Cd68*, T cells by *Cd3e* and leukocytes by *Ptprc*. Two distinct endothelial clusters expressed *Pecam1* and *Cdh5*. Fibroblasts expressed *Myh11* and *Rgs5*. Mature chondrocytes were marked by *Col2a1* and *Acan*, whereas chondroprogenitors were identified by *Col2a1* and *Sox9*. We did not detect a distinct cluster for muscle or satellite cells in this dataset. Next, we questioned whether these populations expressed *Igf1*. *Igf1* expression was restricted to the cambium-layer and fibrous-layer periosteal cells (CL-PCs and FL-PCs) (**Fig. 1 B**). Other cell populations showed negligible expression of *Igf1*. *Igf1r* (encoding for IGF-1 Receptor) was expressed in almost all clusters identified in this dataset. Consistently, CellChat identified the two periosteal cell populations as the dominant senders of IGF-1 (**Fig. 1 C**). The strongest communication probability was observed between FL-PC/CL-PC and the macrophage cluster, indicating that IGF-1 signaling might play a role in a periosteal-immune cell crosstalk during bone homeostasis. We confirmed expression of *Igf1* by RNAScope within the periosteum, as well as the adjacent muscle (**Fig. 1 D**). Together, these data show that in addition to muscle cells, periosteal cells represent a significant source of *Igf1* and play an important role in bone turnover and homeostasis.

Discussion: Here, we show that within bone, *Igf1* expression is restricted to periosteal progenitor populations. CellChat established periosteal cells as the dominant senders of IGF-1, and macrophages and endothelial cells as the receivers of this signal. These findings highlight a potential role for IGF-1 in mediating periosteal cell-immune cell crosstalk within the bone microenvironment. While the scRNA-seq dataset does not distinguish between macrophages derived from the bone marrow and the ones residing within the periosteum, it is possible that IGF-1 signaling may act locally even just within the periosteal niche. This has important implications for our ongoing efforts to prevent CR induced bone loss. We are currently investigating targeted delivery of IGF-1 to skeletal muscle. The observation that both muscle and periosteum express *Igf1* and *Igf1r* suggests that this intervention could prevent both muscle and bone loss during calorie restriction.

Significance/Clinical Relevance: Periosteal IGF-1 represents a local signaling source that may sustain bone formation when systemic IGF-1 is reduced, highlighting new avenues for mitigating bone loss in calorie restriction and related metabolic conditions.

References: ¹Kawai & Rosen *Endo Metab Clin North Am* 2012 ²Bikle+ *Bone* 2015 ³Fontana+ *Aging Cell* 2008 ⁴Liu+ *Dev Cell* 2024 ⁵Nijssure+ *Sci Adv* 2025

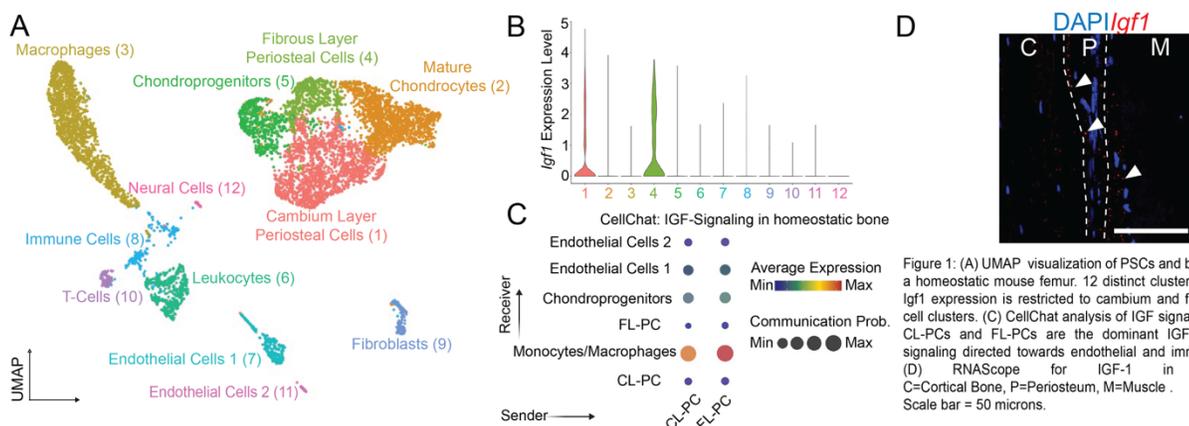


Figure 1: (A) UMAP visualization of PSCs and bone marrow cells from a homeostatic mouse femur. 12 distinct clusters were identified. (B) *Igf1* expression is restricted to cambium and fibrous layer periosteal cell clusters. (C) CellChat analysis of IGF signaling in the periosteum. CL-PCs and FL-PCs are the dominant IGF ligand senders with signaling directed towards endothelial and immune cell populations. (D) RNAScope for IGF-1 in the periosteum. C=Cortical Bone, P=Periosteum, M=Muscle. Scale bar = 50 microns.