Implicating peripheral neural mediated MAPK signaling in obesity induced alterations in bone mass

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INTRODUCTION: Small Peripheral Neuropathy is a common complication in diabetes, affecting around 50% of the diabetic population. Neural dysfunction induced by diabetes-related neuropathy can cause dysregulation of bone metabolism, reduce bone mineral density (BMD), and increase the risk of fractures. At ORS 2021, we showed that high fat diet (HFD) resulted in a periosteal polyneuropathy, which may have contributed to a lower bone mass and bone quality. The periosteum is known to host a niche of bone progenitor and immune cells sensitive to neural ligands. Here, we hypothesized that the lack of innervation in a neuropathic periosteum leads to the dysregulation of the periosteal cellular niche and subsequent bone loss. To investigate this hypothesis, we modeled an innervated periosteum niche bioinformatically to create a communication map between DRGs and periosteum cells. Here, a combination of transcriptomics modeling and *in vitro* assays unveiled essential regulatory signals responsible for long bone cortical homeostasis.

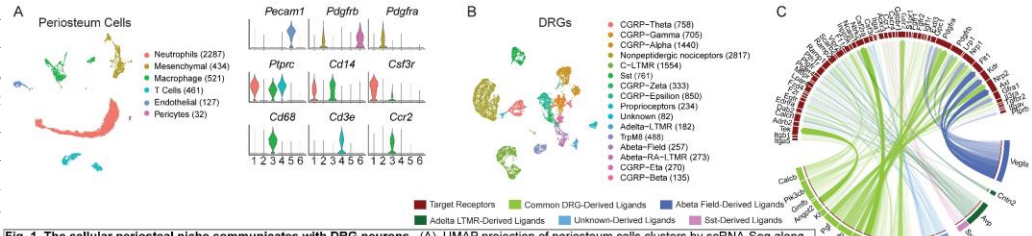

Fig. 1. The cellular periosteal niche communicates with DRG neurons. (A) UMAP projection of periosteum cells clusters by scRNA-Seq along with violin plots of known markers for each cluster. (B) UMAP mouse lumbar dorsal root ganglia (DRG) neurons by scRNA-Seq. (C) Interaction between DRG derived ligands and receptors expressed on stromal cells (MSCs, endothelial cells and pericytes) in normal diet conditions.

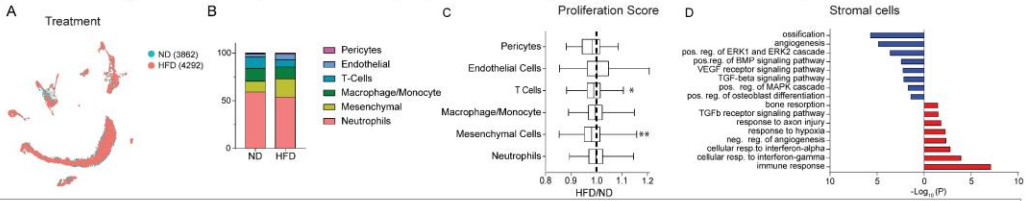

Fig. 2. HFD-induced peripheral neuropathy disturbs the periosteal cellular niche. (A) UMAP visualization by treatment with HFD or ND. (B) Cellular composition of cell cluster based on treatment. (C) Analysis of proliferation score across periosteum cell clusters under ND or HFD. (D) GO term enrichment of the periosteum niche. Module scoring data was analyzed using the Kolmogorov-Smirnov test. *p< 0.05, **p< 0.01, and ***p< 0.001 in comparison to ND control.

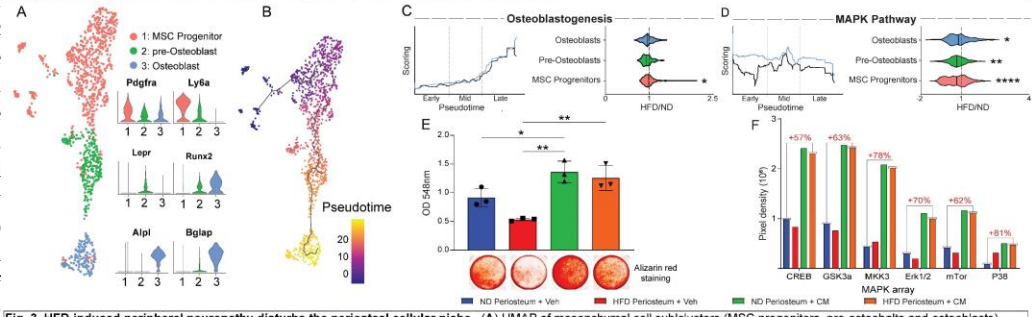

Fig. 3. HFD-induced peripheral neuropathy disturbs the periosteal cellular niche. (**A**) UMAP of mesenchymal cell subclusters (MSC progenitors, pre-osteobalts and osteoblasts) along with known markers for each subcluster. (**B**) UMAP showing the pseudotime trajectory of the mesenchymal cell subclusters. (**C**) Module index scoring of osteoblastogenesis and (**D**) MAPK signaling pathways across pseudotime, and quantification of scores in HFD in relation to ND across each mesenchymal subcluster. (**E**) Osteogenic differentiation assessment by Alizarin red staining in ND and HFD periosteum mesenchymal progenitor cells with/without neural conditioned media. (**F**) Persioteum cells MAPK phosphorylation activation array with/without neural conditioned media. Module scoring data was analyzed using the Kolmogorov-Smirnov test. *p< 0.05, **p< 0.01, and ***p< 0.001 in comparison to ND control.

METHODS: All experiments were conducted under Johns Hopkins University ACUC approval. C57BL/6 animals were purchased for Jackson Laboratories Bar Harbor, Main. HFD feeding was instituted at 4 weeks of age and animals were maintained on normal diet (ND) chow or high fat diet (HFD) chow containing 60% of calories from fat (Research Diets, New Brunswick, NJ; catalogue #D12492) for 12 weeks. Muscles and connective tissues were removed from left femurs and tibias. Bones and DRGs were then digested to isolate periosteum cells and DRG neurons. N=5 male C57BL/6J mice were used per group. DRG neurons were cultured, and their conditioned media (CM) harvested. Periosteum cells were sent to the JHMI Transcriptomics and Deep Sequencing Core. Library preparation was performed using 10X genomics. DRG neuron dataset was obtained from the NCBI database (accession number: GSE154659). Downstream analysis was done using Seurat and Nichenet. Periosteum cells were seeded in 6 well plates for further differentiation and proliferation assays.

RESULTS: scRNA-seq analysis revealed a periosteum niche comprised of 2 compartments: stromal (endothelial cells, pericytes and mesenchymal cells) and immune (neutrophils, macrophage and T cells) (**Fig. 1A**), and 16 DRG clusters (**Fig. 1B**). Interaction analysis between the DRGs and periosteum stromal cells demonstrated a communication of soluble factors and adhesion molecules, including: *Calca, Calcb, Ncam1,Fgf13,Ret,Bdnf and Tac1 (***Fig. 1C**). HFD induced changes in the periosteum niche (**Fig. 2 A-B**) reducing mesenchymal and T cell proliferation (**Fig. 2C**). GO term analysis performed on periosteal stromal cells showed enrichment in immune response and bone resorption under HFD feeding (**Fig. 2D-E**). Further transcriptomic analysis of the mesenchymal cell compartment identified 3 subclusters (MSC Progenitors, pre-osteoblasts and Osteoblast) (**Fig. 3A**). Pathway analysis across pseudotime showed that HFD induced a decreased osteoblastogenesis and MAPK signalization pathways (**Fig. 3C-D**). Finally, *in vitro* differentiation assays on periosteum mesenchymal progenitor cells showed that HFD reduced osteogenic differentiation and MAPK signaling pathway activation. The addition of DRG neural CM significantly enhanced the osteogenic differentiation and MAPK signaling pathway activation of ND or HFD-fed mouse periosteal cells. Remarkably, DRG CM was able to rescue the and osteogenic differentiation potential and MAPK signaling pathway activation of HFD-fed mesenchymal progenitor cells.

DISCUSSION: Here, we showed for the first time a map of the periosteum niche and documented the changes in progenitor cells after HFD. The crosstalk between the periosteum niche and nerves (DRGs neurons) appears to maintain homeostasis through a myriad of cues including soluble factors and adhesion molecules. In HFD conditions, bone is affected leading to changes in progenitor cell phenotype including, MAPK signaling and altered fate differentiation phenotype. Re-exposure to physiological neural input has the potential to restore bone health in obesity related bone disease.

SIGNIFICANCE/CLINICAL RELEVANCE: Metabolic skeletal polyneuropathy is associated with bone architectural deficits, osteogenic differentiation and MAPK signaling in periosteum mesenchymal progenitors, suggesting a direct impact of diabetic neuropathy on bone health.