Trace-n-Seq Unravels Sensory Neuron Control of Heterotopic Ossification

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Introduction

Trauma-induced heterotopic ossification (HO) is a debilitating complication of musculoskeletal injury and is a condition highly relevant to military medicine, as servicemembers are at increased risk of blast-related injuries, high-energy extremity trauma, and repetitive overuse injuries that can predispose to pathologic ossification. Our prior studies demonstrated that sensory nerves regulate aberrant osteochondral differentiation during HO (*Nat.Commun, 2021*). However, our recent single-cell RNA sequencing (scRNA-seq) analyses of dorsal root ganglia (DRGs) highlighted the heterogeneity of peripheral sensory neurons (*Science, 2026*). These observations raise key unresolved questions: which specific sensory neuron populations innervate HO lesions, and how do these neurons respond to HO injury to regulate its genesis?

Methods

All animal experiments were conducted under approval of the Johns Hopkins IACUC. To identify HO-innervating sensory neurons, AAV-PHP.S-tdTomato, an engineered viral vector with tropism for peripheral neurons, was injected into the HO site to achieve retrograde labeling of DRG neurons. After 4 weeks of labeling, mice underwent burn/tenotomy surgery to induce HO. To define dynamic neuronal responses during HO progression, labeled DRGs were analyzed by scRNA-seq at 0-, 7-, and 21-days post-injury, corresponding to key stages of HO progression. Sequenced single cell reads were aligned to a mouse reference transcriptome (GRCm39-2024-A) using Cell Ranger 8.0.1. Output files were analyzed using Seurat.V5.

Results

scRNA-seq recovered 5,493 neurons enriched for CGRP-expressing nociceptive and mechanosensory populations. Temporal transcriptomic analysis revealed dynamic neuronal responses during HO progression related to cell differentiation, angiogenesis and immunoregulation. Cell-cell interaction analysis further profound early changes in nerve-to-HO signaling corresponding to the inflammatory phase. Two independent interactome analyses convergently identified nerve-derived signaling as targeting HO formation, including PTN (Pleiotrophin) and FGF (Fibroblast Growth Factor) pathways.

Discussion

Molecular mapping of sensory neurons that innervate HO are primarily nociceptive and mechanoreceptive nerves. This transcriptomic map implicates key neural-derived signaling pathways in early tissue injury that may be targeted to prevent HO formation. These results define a new framework for understanding HO pathogenesis and suggest targeting sensory nerve-derived signaling represents a strategy to reduce HO and optimize return to duty.

Figure 1. Identification and characterization of tendon-innervating sensory neurons during HO

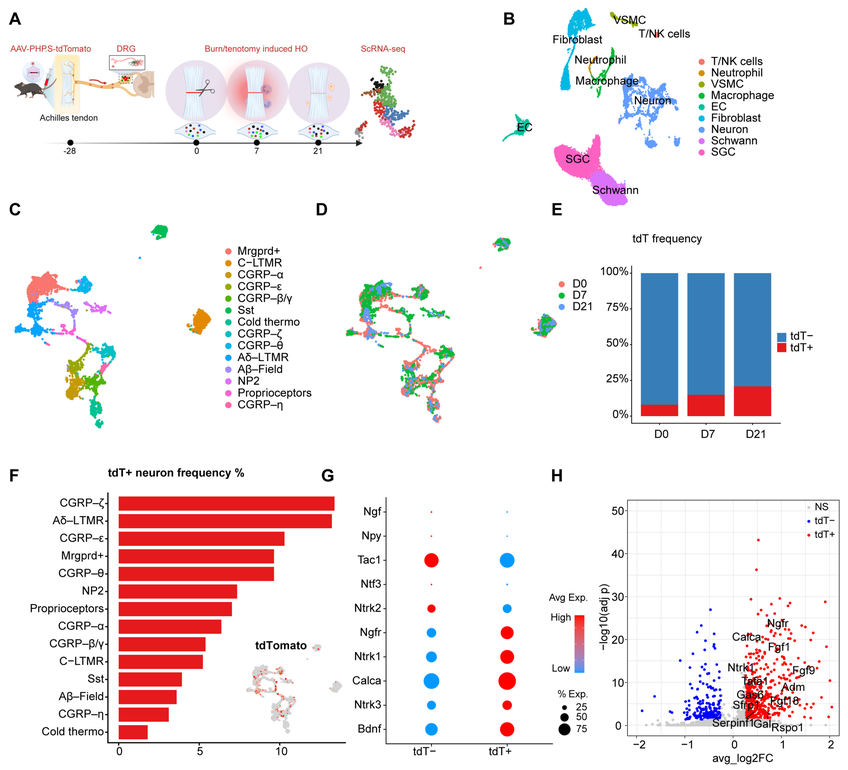

Figure 1. Identification and characterization of tendon-innervating sensory neurons during HO (A) Experimental schematic. Achilles tendon–innervating sensory neurons were retrogradely labeled by injection of AAV-PHP.S-tdTomato into the Achilles tendon 28 days before burn/tenotomy injury. Dorsal root ganglia (DRGs) were harvested at day 0, day 7, and day 21 after injury and processed for single-cell RNA sequencing. **(B)** UMAP visualization of all DRG cells, annotated into major cell populations, including neurons, Schwann cells, satellite glial cells (SGCs), fibroblasts, endothelial cells, vascular smooth muscle cells (VSMCs), macrophages, neutrophils, and T/NK cells. **(C)** UMAP visualization of neuronal cells classified into sensory neuron subtypes based on established transcriptional markers. **(D)** Distribution of neuronal cells from the day 0, day 7, and day 21 time points within the integrated neuronal UMAP . **(E)** Relative frequencies of tdTomato-positive (tdT+) Achilles tendon–innervating neurons and tdTomato-negative (tdT−) neurons at each time point. **(F)** Percentage of tdT+ cells within each sensory neuron subtype. The inset shows tdTomato expression across the neuronal UMAP. **(G)** Dot plot comparing the expression of neurotrophins, neurotrophin receptors, and neuropeptides between tdT− and tdT+ neurons. Dot size represents the percentage of cells expressing each gene, and color indicates average expression. **(H)** Volcano plot showing differentially expressed genes between tdT+ and tdT− neurons. Neuronal secreted HO related differentially expressed genes are labeled. gray points indicate genes that were not significantly differentially expressed.

Figure 2. Temporal transcriptional programs in Achilles tendon–innervating neurons following burn/tenotomy injury.

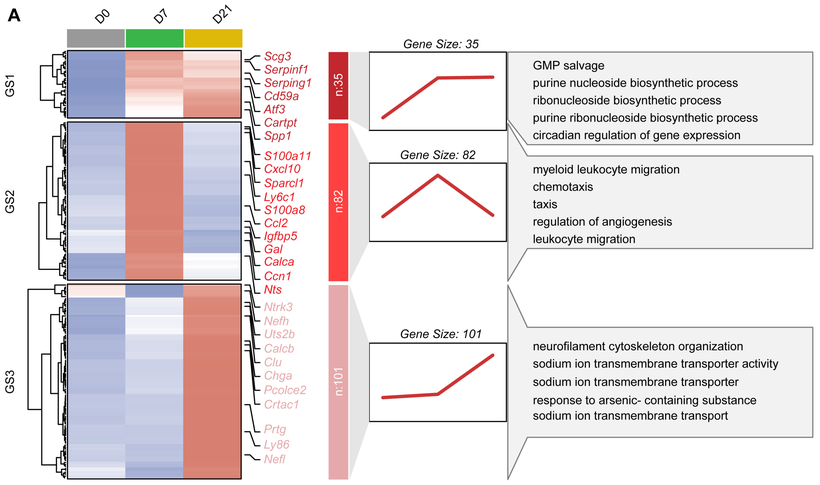

Figure 2. Temporal transcriptional programs in Achilles tendon–innervating neurons following burn/tenotomy injury. (A) Gene sets (GSs) in tdT+ neurons were identified by Mfuzz based on their dynamic expression patterns at day 0 (D0), day 7 (D7), and day 21 (D21) after injury. Upregulated genes were grouped into three gene sets according to their temporal expression patterns. Representative genes are indicated beside the heatmap. Line plots show the average expression trajectory for each gene set, and top5 Gene Ontology terms associated with each set are shown on the right.

Figure 3. Dynamic neuron-to-tissue communication during HO

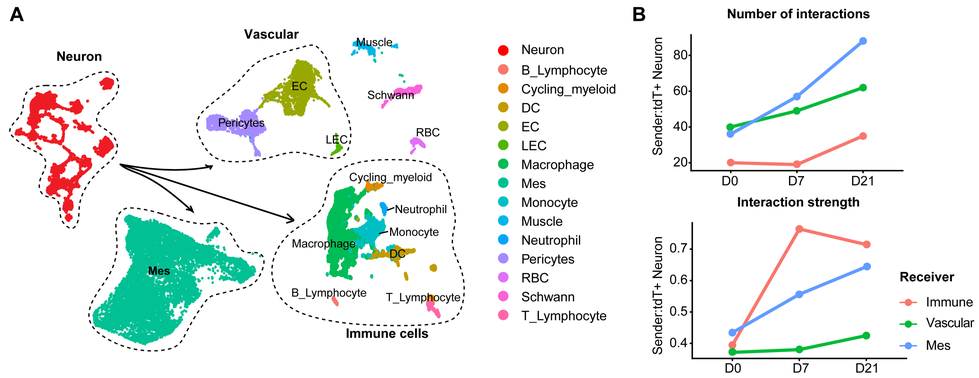

Figure 3. Dynamic neuron-to-tissue communication during heterotopic ossification. (A) Integrated UMAP showing major neuronal, mesenchymal, vascular, immune, and other cell populations. Arrows indicate inferred signaling from tdTomato-positive (tdT+) tendon-innervating neurons to mesenchymal, vascular, and immune receiver populations. **(B)** CellChat analysis showing changes in the number and overall strength of neuron-derived interactions with each receiver population at days 0, 7, and 21 after injury.

Figure 4. Dynamic cell–cell signaling from Achilles tendon–innervating neurons during HO

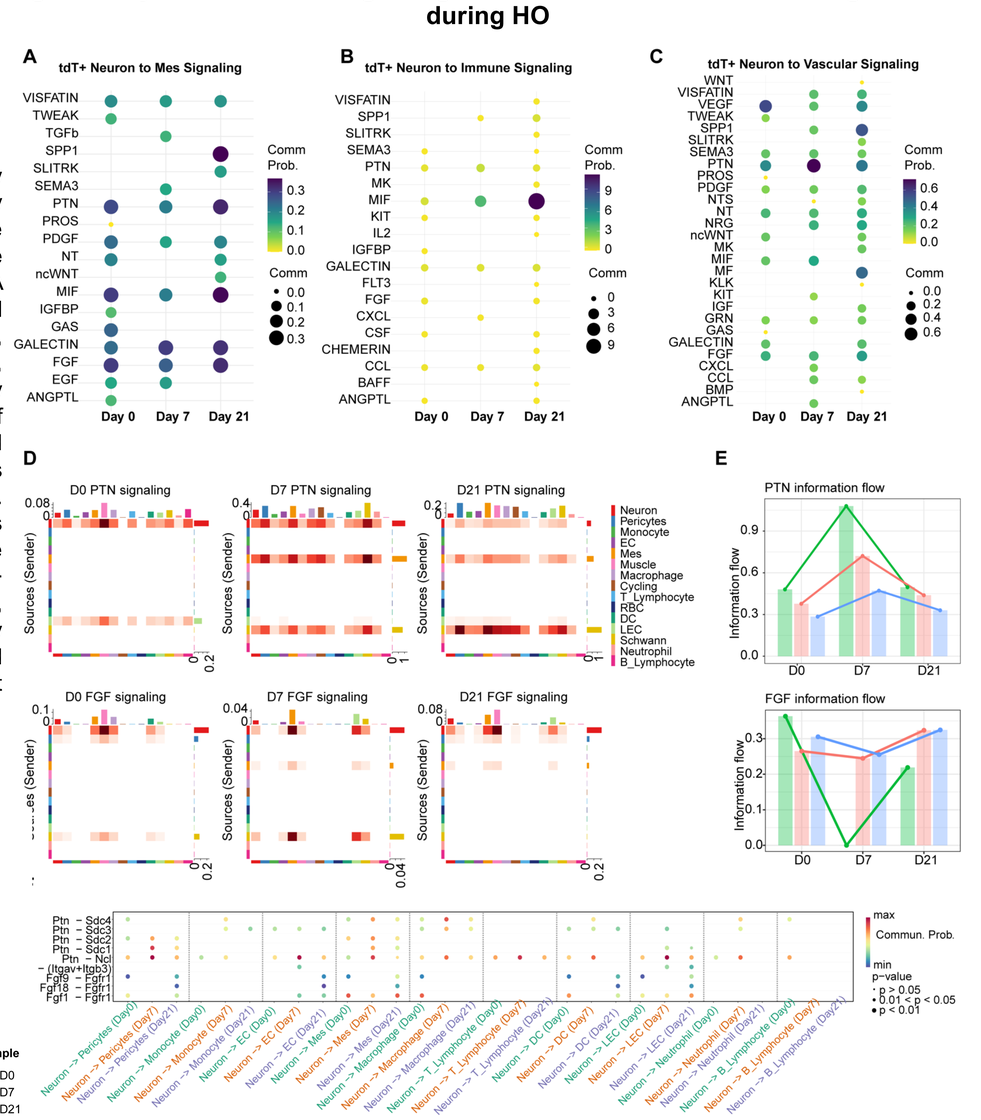

Figure 4. Dynamic cell–cell signaling from Achilles tendon–innervating neurons during heterotopic ossification. (A–C) CellChat analysis of signaling pathways from tdTomato-positive (tdT+) neurons to mesenchymal **(A)**, immune **(B)**, and vascular **(C)** cells at days 0, 7, and 21 after injury. Dot size and color indicate communication probability. **(D)** Heatmaps showing PTN and FGF signaling networks among major cell populations at each time point, with sender populations shown in rows and receiver populations in columns. **(E)** Changes in PTN and FGF information flow from tdT+ neurons to immune, vascular, and mesenchymal cells over time. **(F)** Predicted PTN- and FGF-associated ligand–receptor interactions between tdT+ neurons and individual receiver populations. Dot color represents communication probability, and dot size indicates statistical significance