Acute Transcriptomic Response of Synovium and Dorsal Root Ganglia to Joint Injury

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INTRODUCTION: The synovium undergoes marked structural and cellular changes during osteoarthritis (OA). Synovitis is a consistent correlate to OA pain [1,2], but the mechanisms underlying synovial-derived pain remain incompletely understood. Emerging clinical evidence suggests crosstalk between synovial cells and knee-innervating dorsal root ganglia (DRG) neurons drives nerve sprouting and pain sensitization in the context of synovial disease [3,4]. In a non-surgical mouse model of ACL rupture (ACLR), we observe progressive knee hyperalgesia and rapid synovial nociceptor sprouting as early at 1-week post-injury [5,6] – this preempts major structural changes associated with post-traumatic (PT)OA, suggesting nociceptor sprouting is part of the acute post-injury response. To elucidate the signaling axes underlying this response, we utilized RNA transcriptomics to (1) characterize neurotrophic transcriptional signatures activated in synovium and DRG following ACL injury, and (2) model the key injury-enriched signaling hubs underpinning synovium-nerve crosstalk.

METHODS: Under IACUC approval, 12-week-old male C57Bl/6 mice underwent ACLR or sham procedure. At 1-, 3-, or 7-days post-injury, paired ipsilateral synovium and knee-innervating L3-L5 DRGs were harvested and processed for bulk RNAseq (n=6/condition) or single-cell (sc)RNAseq (n=10 pooled to 1 rep/condition). Bulk RNAseq data were analyzed for differentially-expressed genes (DEGs) via weighted Limma-Voom, and pathway enrichment via gene-set enrichment analysis (GSEA). scRNAseq (>50K cells/tissue) was annotated via published annotations [6-8]. To define a putative synovium → nerve interactome, we screened a DRG ligand-receptor database [9] to identify interactions between ACLR-enriched synovial ligands and DRG neuron receptors.

RESULTS: The synovium exhibited a marked, transcriptome-wide response to ACLR, which gradually evolved from 1-7d post-injury (Fig 1A). Rapid activation of pathways related to neuroinflammation and broad immune activation was observed, including activation of early responders such as neutrophils and macrophages, with peak enrichment observed at 1-3d and incomplete resolution by 7d post-injury (Fig 1B). Enrichment of pathways related to nerve sprouting and angiogenesis, including terms related to neuron projection development and axon guidance, were more progressively activated, with peak enrichment at 7d post-injury (Fig 1B). DRG response to ACLR was comparatively subtler at the gene-level (Fig 1A), but significant enrichment of several neurotrophic pathways (including axonogenesis, dendrite development, and neuron projection extension) was observed across all timepoints, with different pathways reaching peak enrichment at 1-, 3-, and 7d post-ACLR, suggesting an evolving response spanning neuron sprouting, development, and maturation (Fig. 1B). We generated a putative interactome comprising 33 ACLR-enriched synovial ligands interacting with 15 DRG neuronal receptors (Fig 1C). Major signaling hubs included known neurotrophic and axon guidance pathways, including nerve growth factor (*Ngf*) interacting with both its known receptors (TrkA (*Ntrk1*) and p75NTR (*Ngfr*)), a broader family of proteins known to modulate NGF signaling (*Nrp1, App, Clstn1*), semaphorin-plexin signaling (*Sema3a, b, d, e, f* interacting with *Plxna4*, *Nrp1*), and *Slit3-Robo2* signaling. Several growth factor families were predicted with known roles regulating stromal and neuronal lineages, including FGF (*Fgf 2, 7, 18* interacting with *Fgfr1, Nrp1, Cd44*) and BMP-2 signaling (*Bmp2, Fst* interacting with *Bmp2rl*); similarly, chemokine and cytokine-related factors (*Cxcl12*, *Chl1, Lgals3bp, Clcf1*) also have predicted interactions with DRG neuronal receptors (*Itgb1, Cntfr, Il6st, Lifr, Nrp1*), suggesting that DRG neurons may be able to directly sense changes in these synovial-derived stromal and inflammatory factors. Utilizing our scRNAseq data, we unbiasedly clustered predicted ligands into 5 distinct gene modules, each associated with specific cell types, and enriched across different timescales (Fig 1D). The two largest modules (2,3) were primarily expressed by fibroblasts, including most Sema and FGF ligands, and peak expression of these modules was observed at 3-7d post-injury; conversely, vascular cells (pericytes, endothelial cells) exhibited acute (1d) upregulation of two modules (1,4) including NGF and BMP-2 signaling. Within the DRG, expression of most receptors was primarily associated with *Calca+/Tac1+* peptidergic nociceptors, including receptors for NGF, Sema, FGF, and BMP-2 (data not shown). Preliminary analysis of Xenium 5K spatial transcriptomics data (Fig 1E) demonstrates the potential to extend this analysis to localize expression of neuroactive factors within specific spatial niches (e.g. *Sema3a* in the synovial lining, *Fgf7* in the sublining, *Plxna4* receptor expression in *Calca+* peptidergic DRG nociceptors).

DISCUSSION: To our knowledge, this is the first description of acute changes in paired synovium and DRG tissues following joint injury. Our interactome analysis identified several signaling hubs and the specific cell types potentially facilitating this communication. These results clearly suggest that development of neuroplasticity and pain following ACL injury is underpinned by a complex network of evolving crosstalk axes between the synovium and DRG (Fig 1F). Ongoing work seeks to provide protein-level validation of these *in silico* findings, and to refine this list further to a short list of potentially druggable targets.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the specific signaling axes underpinning pathologic synovium-nerve crosstalk is a key step in developing targeted therapies that mitigate the risk of synovial neoinnervation and synovial-derived OA pain following joint injury.

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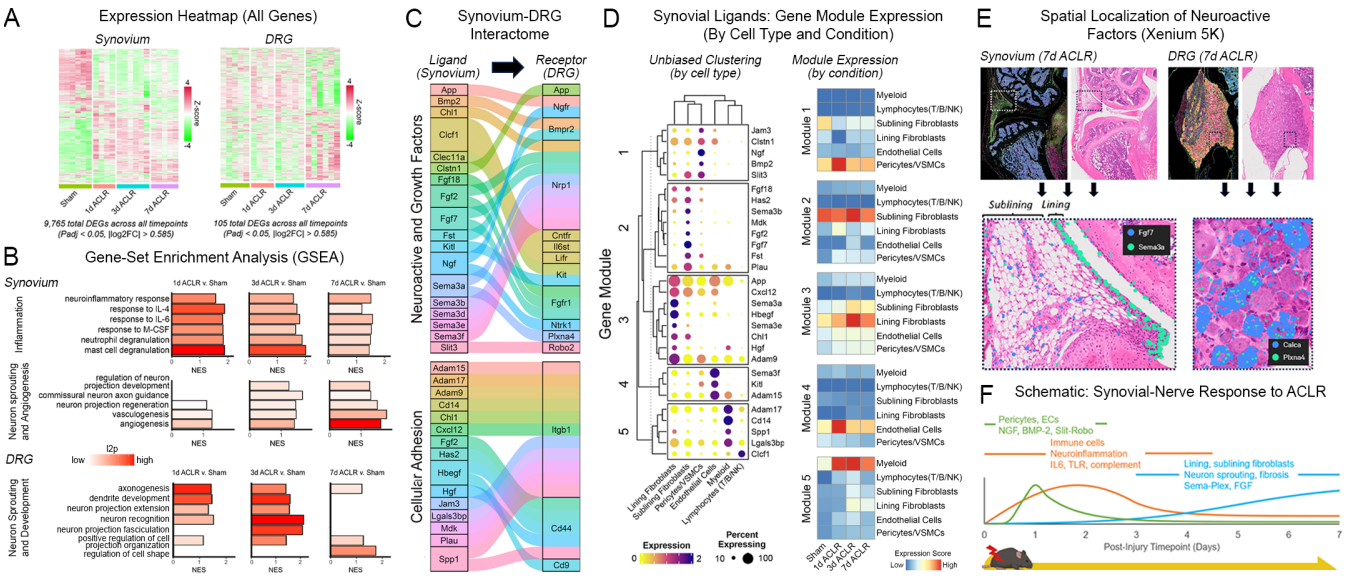

Figure 1. *(A) Bulk RNAseq response to ACLR. (B) ACLR-enriched pathways. (C) Synovium-nerve interactome (alluvial plot). (D) Synovial ligand expression by cell type and timepoint. (E) Xenium 5K localization of neuroactive ligands and receptors. (F) Schematic of time- and cell type-dependent response to ACLR.*