Single-cell analysis reveals antioxidant programs associated with post-traumatic osteoarthritis resistance and highlights candidate therapies

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Abstract

Post-traumatic osteoarthritis (PTOA) develops after joint injury and disproportionately affects military personnel. Despite its prevalence, no disease-modifying therapies exist, partly because the early molecular mechanisms driving disease remain poorly understood. We compared PTOA-resistant MRL/MpJ (MRL) and susceptible C57BL/6J (BL6) mice using histology, single-cell RNA sequencing (scRNA-seq), and proteomic profiling to define protective cellular and molecular programs and identify candidates for drug repurposing. We identified antioxidant programs associated with PTOA resistance and highlight new candidate therapies based on drug repurposing.

Materials and methods

Male BL6 and MRL mice underwent tibial compression injury at 10 weeks (W) of age to induce anterior cruciate ligament rupture. Knee joints were collected at baseline (D0) or 4W post-injury. Histology, immunostaining, proteomics, and scRNA-seq of CD45$^-$ joint cells were performed. Differential gene expression, pathway enrichment, and redox pathway scoring were used to compare strain-specific responses to injury. Chondrocyte gene expression signatures were queried against the Connectivity Map (CMap), a database of drug-induced transcriptional profiles [1, 2], to identify drugs predicted to reverse PTOA-associated molecular changes.

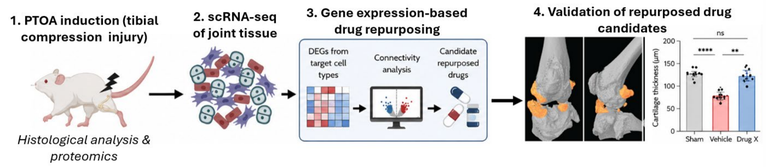

***Figure 1*: Experimental workflow**. *Histological and multi-omic analyses of knee joints collected at D0 and 4W after injury identified protective responses. Chondrocyte gene signatures were queried against CMap to identify candidate repurposable therapies for in vivo validation.*

Results

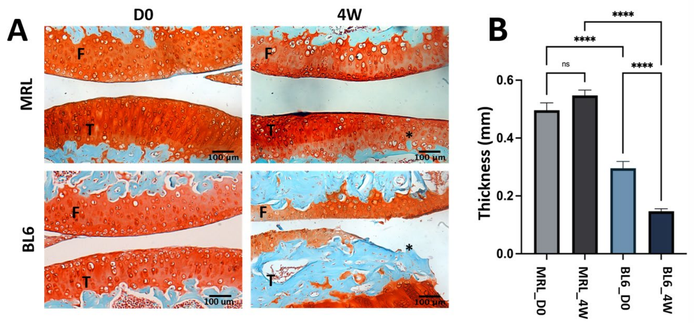

***Figure 2. MRL mice are protected from PTOA-induced cartilage degeneration**. **A**) Safranin O/Fast Green staining shows preserved cartilage in MRL mice compared with severe degeneration in BL6 mice 4W after injury. Scale bar = 100 μm. **B**) Quantification of cartilage thickness (mean ± SEM). *p < 0.05, **p < 0.01, ****p < 0.0001; ns, not significant.*

Single-cell RNA sequencing identifies cell type-specific changes in MRL and BL6 knee joints after injury

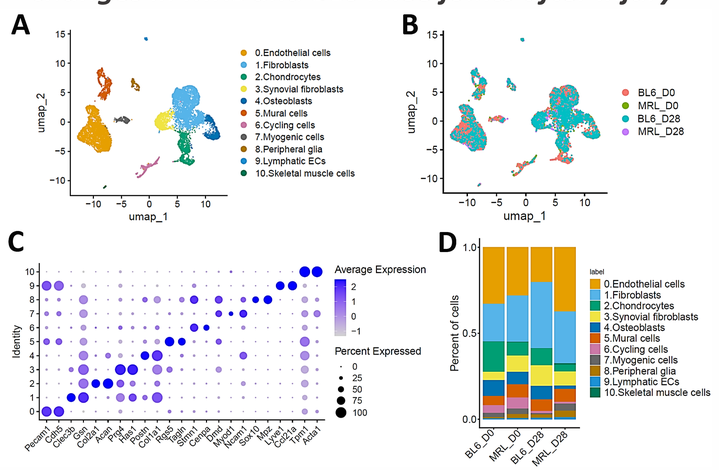

***Figure 3. scRNA-seq analysis of knee joint cells filtered of immune cells. A**) UMAP of merged non-immune cells from MRL and BL6 knee joints. **B**) UMAP colored by experimental group. **C**) Canonical marker gene expression identifies major cell populations. **D**) Cell type proportions across groups reveal significant injury-induced expansion of fibroblasts and synovial fibroblasts and loss of chondrocytes in BL6, while MRL joints exhibit only mild to moderate compositional changes.*

Gene expression programs associated with PTOA resistance

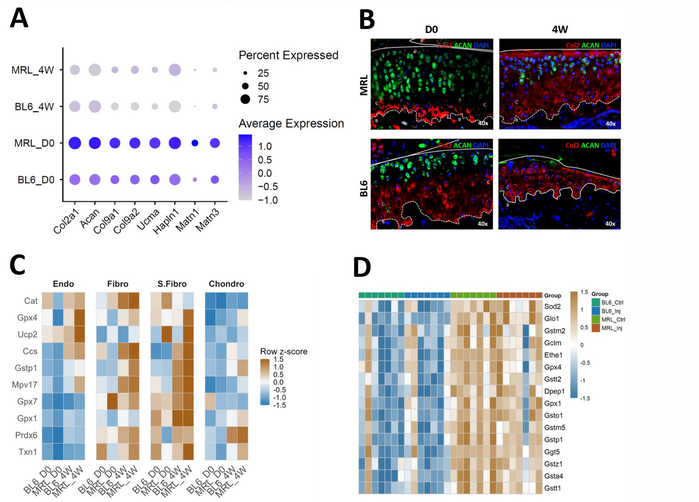

***Figure 4. MRL display a constitutively elevated antioxidant program. A)** Cartilage matrix gene expression. **B)** COL2A1/ACAN immunofluorescence. **C**) Antioxidant gene expression across key cell types (Endo: endothelial; Fibro: fibroblasts; S. Fibro: Synovial fibroblasts; Chondro: chondrocytes). **D)** Proteomic profiling of whole knee joints confirms elevated antioxidant proteins in MRL vs. BL6.*

Gene expression signature-based drug repurposing and in vitro validation

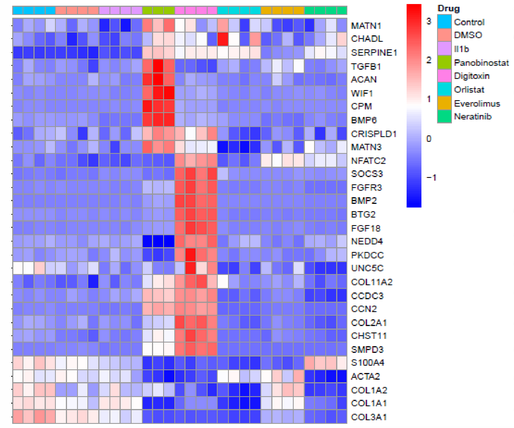

***Figure 5. Gene expression signature–based drug repurposing and in vitro validation.** Differentially expressed genes between MRL and BL6 chondrocytes were queried against CMap to identify compounds predicted to reverse PTOA-associated molecular changes. Candidate compounds were evaluated in IL-1β-stimulated chondrocytes. **Panobinostat** and **digitoxin** most effectively induced cartilage-associated gene expression, supporting their therapeutic potential.*

Validation of repurposed drug candidates in vivo

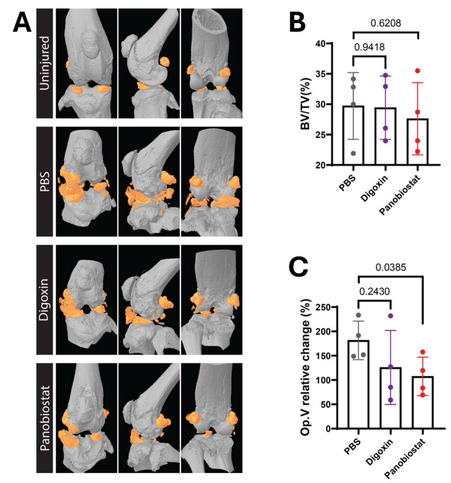

***Figure 6. Experimental validation of repurposed drug candidates. A)** Representative microCT reconstructions of knee joints showing osteophytes (orange). **B**) Subchondral bone volume fraction (BV/TV) relative to uninjured mice. **C)** Osteophyte volume relative to uninjured controls. Panobinostat significantly reduced osteophyte formation, a hallmark of PTOA, whereas Digitoxin showed a similar but non-significant trend. Histological evaluation is ongoing.*

Conclusions and future directions

- Protective pathways in MRL mice reveal therapeutic targets for PTOA.
- scRNA-seq–guided drug repurposing identifies promising candidates.
- Future work will define the mechanisms underlying the protective MRL phenotype and evaluate candidate therapeutics in long-term preclinical studies.

***References:** Lamb et al, Science. 2006**;** Subramanian et al, Cell, 2017*

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