Annulus Fibrosus Progenitor Gene Signature is Conserved in Human, Bovine, and Mouse Intervertebral Discs

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INTRODUCTION: Pathologies of the intervertebral disc (IVD) are major drivers of the global disability and healthcare burden associated with back pain. Further, annulus fibrosus (AF) defects are an IVD degeneration phenotype that contribute directly to tissue herniation, inflammation, and fibrosis [1]. Current Phase 2/3 clinical trials of IVD-targeted cell therapies delivering stem- or nucleus pulposus-derived cells demonstrate promising pain reduction, but other patient-reported outcomes show inconsistent improvements [2, 3], likely reflecting insufficient healing in the absence of clearly defined tissue-specific progenitors. Last year, we defined transcriptomic signatures for bovine AF progenitors (AFPs) using single-cell RNA-sequencing (scRNA-seq) and identified cell culture conditions that enriched this phenotype [4]. This year, we sought to investigate whether this AFP transcriptomic signature is conserved across species by analyzing mouse and human scRNA-seq datasets, and evaluated its preclinical and clinical relevance by comparing similarity across species. Therefore, the aims of this study were to: (1) identify AFPs in mouse and human outer AF cells using transcriptional entropy profiling and (2) define an AFP gene list that is conserved across species using a normalized framework for cross-species transcriptomic comparisons.

METHODS: scRNA-seq datasets from bovine (bIVD) [4], mouse (mIVD) [5], and human (hIVD) [6] IVDs were processed independently using the Seurat pipeline. Outer AF populations were identified and reclustered, followed by visualization with uniform manifold approximation and projection (UMAP) and differential gene expression analysis. Transcriptional entropy profiling with the pySCE library identified putative stem/progenitor populations, and a previously defined bovine AFP population was used as a reference for cross-species comparisons [4, 7]. The Jaccard coefficient is commonly used to quantify similarity between two sets [8], but to account for interspecies differences in transcriptome size and baseline similarity, we divided the Jaccard coefficient between bovine AFP genes and mouse or human clusters by the global transcriptome overlap for each species pair, yielding a normalized metric for cluster-level similarity. Like other enrichment tools, statistical significance was assessed using a hypergeometric test with Benjamini-Hochberg correction [9]. A Venn diagram illustrated the intersection of DEGs from the top entropy-scoring clusters.

RESULTS: UMAP clustering of bovine (17,454), mouse (3,116), and human (1,703) oAF cells resolved 18, 8, and 4 transcriptionally distinct subpopulations, respectively. DEGs from each subpopulation were carried forward for downstream cross-species comparisons. Among mouse and human oAF clusters, mIVD Cluster 3 and hIVD Cluster 1 exhibited significantly greater transcriptional entropy scores, identifying them as AFP populations (Fig 1). Consistently, these same clusters showed the highest normalized similarity scores relative to the bovine AFP population (Fig 2), indicating strong interspecies conservation between the top entropy-scoring clusters. Comparison of DEGs across bovine, mouse, and human AFPs revealed a shared gene signature of 38 conserved features (Fig 3), including the canonical stem cell-associated marker for nucleostemin, *GNL3*.

DISCUSSION: This study demonstrated the presence of AFP populations across bovine, mouse, and human IVDs, as revealed by the transcriptional entropy profiling that identified mIVD Cluster 3 and hIVD Cluster 1 as AFPs. Our normalized similarity scoring framework confirmed mIVD Cluster 3 and hIVD Cluster 1 as the most transcriptionally conserved relative to bovine AFPs, while all other comparisons showed markedly lower, non-significant scores, reinforcing that cross-species conservation is restricted to the top entropy-scoring populations. Within the conserved AFP gene set, *GNL3* was particularly notable given its established role in regulating proliferation and stemness of human mesenchymal stem cells, as well as its expression in rabbit-derived AF stem-like cells [10]. Additional conserved features, including *CNOT3, SAFB, EIF3A,* and *TRIM27*, further suggest a progenitor phenotype with functional programs linked to RNA processing, ribosome biogenesis, and epigenetic regulation of differentiation. Beyond future immunostaining validation of these markers, the lack of clear extracellular surface markers in this AFP gene set underscores the need to identify generally expressed surface markers for downstream multi-selection sorting. Functional perturbation studies with gene-edited *in vitro* systems and loss- or gain-of-function *in vivo* models will be critical to establish the regenerative potential of AFPs for AF repair. In conclusion, this study determined that a novel AFP transcriptomic signature can be identified in bovine, mouse and human IVDs with 38 genes conserved across all species.

SIGNIFICANCE: Our results demonstrate translational relevance of pre-clinical bovine and mouse IVD models because of their similarity to human AFPs, and advance the development of *in vitro* and *in vivo* AFP-mediated regenerative strategies for human IVD degeneration.

REFERENCES: [1] Hartvigsen+ *Lancet* 2018, [2] Gornet+ *IJSS* 2024, [3] Beall+ *Spine J* 2025, [4] Rodriguez+ *ORS* 2025; [5] Jacobsen+ *ORS* 2025, [6] Jiang+ *iScience* 2022, [7] Charytonowicz+ *Nat Comm* 2023, [8] Xie+ *IntroSysBioinf* 2025, [9] Xie+ *Curr Protoc* 2021, [10] Guo+ *Methods Mol Biol* 2018

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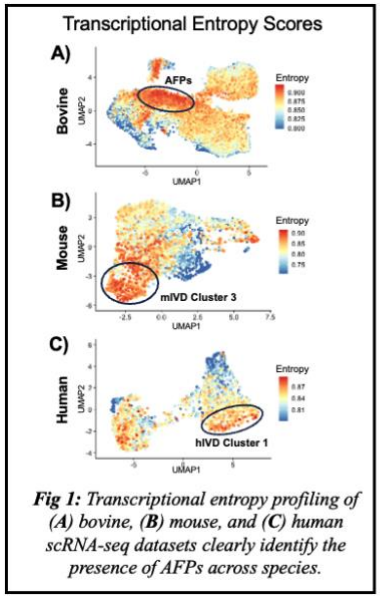

Fig 1: Transcriptional entropy profiling of (A) bovine, (B) mouse, and (C) human scRNA-seq datasets clearly identify the presence of AFPs across species.

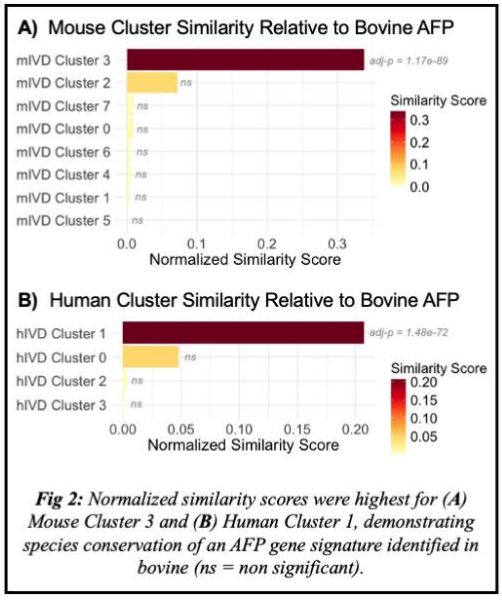

Fig 2: Normalized similarity scores were highest for (A) Mouse Cluster 3 and (B) Human Cluster 1, demonstrating species conservation of an AFP gene signature identified in bovine (ns = non significant).

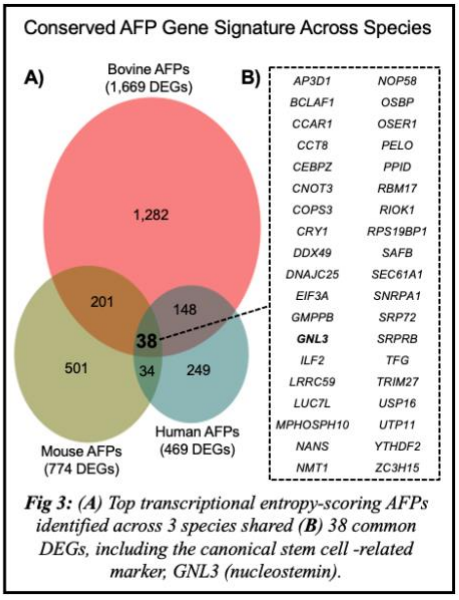

Fig 3: (A) Top transcriptional entropy-scoring AFPs identified across 3 species shared (B) 38 common DEGs, including the canonical stem cell -related marker, GNL3 (nucleostemin).