Development of a Comprehensive Intervertebral Disc Degeneration Large Animal Model

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Introduction: Lower back pain (LBP) is one of the most common medical complaints in the US with up to 40% of lower back pain cases attributed to intervertebral disc (IVD) degeneration. LBP has been extensively studied using small animal models such as mice, rat, and rabbits which, due to their small size and well-established biobehavioral tests for pain, are excellent models for the development of both IVD degeneration and back pain. However, their small size makes less relevant for human disease and the development of new therapies may yield false-positive outcomes. Larger animal models can more accurately model what occurs in humans, however, there is not a well-established method of measuring the development of low back pain in large animals.

The purpose of this study is to develop a robust method and comprehensive of quantifying pain stemming from IVD degeneration via porcine biobehavioral testing coupled with novel MR imaging that was previously validated in pigs and humans. We will also be applying a multi-omics approach to further elucidate the mechanisms driving IVD degeneration in large animal at the molecular and cellular levels.

Methods: IVD degeneration was induced in 2–4-month-old Yucatan minipigs (n=6) using a 16G needle stick injury to the L3-L4, L4-L5, and L5-L6 intervertebral discs (Fig 1A). Wind up ratio and Glasgow pain scale were performed every two weeks starting at baseline until sacrifice to evaluate and quantify the development of pain. Every four weeks pigs underwent MRI imaging to monitor the development of IVD degeneration using the traditional and novel qCEST (K_sw) sequences. At 16 weeks post injury, pigs were sacrificed and IVD were collected for single cell RNA sequencing (scRNA-seq) and histology.

Results: Macro images shows significant degeneration at harvest (Fig. 1B). MRI imaging of the injured discs at week 4 showed significant decrease in T1 which represents longitudinal relaxation time, T2 which represents transverse relaxation time, and MTR which indirectly quantifies glycosaminoglycan content (Fig. 1C). K_sw, which our group has previously validated as directly correlated to tissue pH, was found to be higher in the injured discs starting at week 4 and continuing up to harvest at 16 weeks. The Glasgow test showed the development of pain responses starting at week 10 with pain scores continuing to increase up to 16 weeks (Fig. 2A). Wind up ratio which indicates increased responsiveness to repeated noxious stimuli, showed a significant increase in sensitivity in the dermatomes associated with the injured discs, starting 4 weeks post injury (Fig 2B). Furthermore, the injured area had higher average PEPS scores than the areas outside the injured disc's dermatome, suggesting pain development in the specific area of interest only. Preliminary scRNA sequencing of the nucleus pulposus showed the development of unique clusters in the injured NP (clusters 8 and 11) and a loss of clusters (clusters 0, 1 and 2, Fig. 3A-B). Analysis of the clusters has identified several markers that are unique to each cluster (Fig. 3C). Preliminary analysis of cluster 8 has identified its highest expressing genes as being associated with apoptosis and fibrosis (Fig. 3D). Histological staining of the tissue showed significant loss of NP tissue and increased tissue density at 12 weeks post injury (data not shown).

Discussion: This study demonstrates the potential of a novel method of quantifying pain in a porcine model. MR Imaging showed gradual development and significant degeneration of the IVD post injury. Biobehavioral testing demonstrated a significant increase in pain behavioral response from the pigs compared to pre-injury baseline. scRNA-seq showed significant changes between the degenerated injured IVD and the healthy one, namely in the development of unique clusters in each of the samples. Histology demonstrated significant degeneration of the disc post injury. Additional analysis must be performed to further elucidate the molecular signatures of disc degeneration on the single cell level.

Significance / Clinical Relevance: Development of a pain assessment system can help us determine how to better treat and prevent lower back pain from occurring. Establishment of a pain assessment system in large animal models can help to accelerate promising pre-clinical treatments towards the clinic.

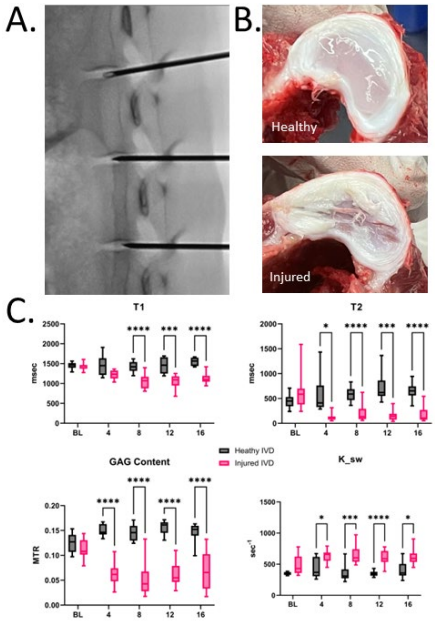

Fig 1: Injury and MR imaging. *(a) X-ray imaging of induction of IVD injury. (b) Analysis of MR imaging showed, decreased T1 and T2, glycosaminoglycan content, and increased pH within the injured discs measured by qCEST sequence. X axis – Weeks, Bars - SE, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

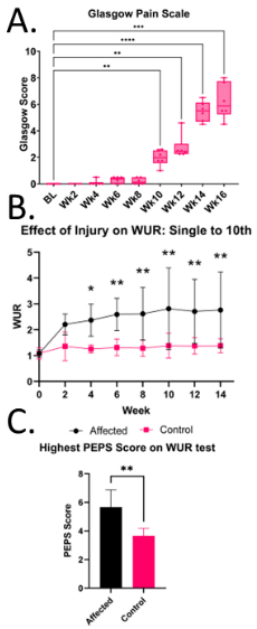

Fig 2: Biobehavioral testing shows significant pain response following injury. *(a) Glasgow pain scale results. (b) Wind up ratio, (c) PEP score. Bars - SE, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

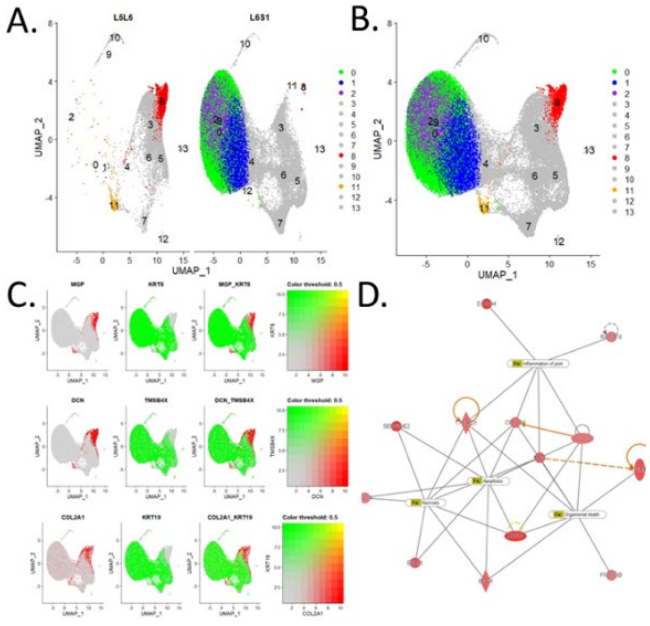

Fig 3: scRNA-seq identified a distinct cell type (Cluster 8) that was found almost exclusively in the injured disc. *Clusters of interested highlighted by color with (a) separated samples. (b) samples combined, (c) Expression of select markers of interest by cluster location, (d) IPA map identifying associated morbidities associated with those molecular signatures.*