

Transcriptomic Profiles of Chronic Pain and Disability in Patients with Intervertebral Disc Pathology

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INTRODUCTION: Low back pain (LBP) is the leading cause of disability with intervertebral disc (IVD) degeneration being the most common cause¹. Diagnoses such as spinal stenosis and disc herniation also contribute significantly to the population of LBP sufferers^{2,3}. Prior studies have shown correlations between severity of spine pathology and pain. To investigate potential mechanisms contributing to the development of painful disc pathology, our group has previously characterized levels of inflammatory cytokines and chemokines in the blood of this patient population. Inflammation was observed in all diagnoses compared to control subjects, with cytokine and chemokine levels increasing with disease severity⁴. However, how inflammation and the immune response contribute to the development of pain and disability in patients is unknown. Whole blood transcriptomic analysis has been used previously to reflect genetic changes in the nervous system making it an attractive approach for unbiased characterization of pain. One study used this technique to compare acute and chronic LBP to healthy subjects, but pain in this study was not attributed to spine pathology. Additionally, the relationship between pain and disability was not analyzed⁵. The **objective** of this study was to characterize the global transcriptomic changes associated with chronic pain and disability in the blood of patients with IVD pathology using bulk RNA sequencing. The **hypothesis** was that patients experiencing chronic pain and disability validated using patient-reported outcomes (PROs) will exhibit transcriptomic changes in inflammation and the innate immune response that are dependent on the severity of pain and disability. **METHODS:** This study was approved by the Institutional Review Board (IRB). **Patient Data Collection:** Subjects (n=29) were recruited from patients being evaluated for epidural steroid injections (ESI) in the lumbar spine region and informed consent was obtained. All subjects were 18 years or older and had a diagnosis of disc herniation, spinal stenosis, and/or disc degeneration. Patients were excluded if they had a prior history of lumbar spine surgery, previous ESIs in the last 6 months, or a history of other inflammatory or oncological conditions. Data collected from LBP subjects included demographic information, duration of symptoms, history of smoking, diabetes, depression, diagnosis, and social security disability insurance (SSDI) status or workers' compensation status. Demographic data included gender, ethnicity, race, age, and BMI. PROs collected were pain level using a 10-point visual analog scale (VAS) and disability level using the Oswestry disability index (ODI), with both VAS and ODI categorized as low (VAS≤6, ODI≤40) or high (VAS>6, ODI>40). A simple linear regression was performed between paired ODI and VAS scores using an Analysis of Covariance (ANCOVA) to determine significance. **Blood Sample Collection and Processing:** Blood samples were collected at baseline prior to ESI treatment using venipuncture into a BD Paxgene RNA tube before storage and RNA isolation according to the manufacturer's protocol. **Bulk RNA Sequencing:** RNA quality was validated using an Agilent Bioanalyzer 2100 with only samples with RNA Integrity Number (RIN) of greater than 7.4 used for sequencing. RNA sequencing was then performed by the Genome Center using a STRPOLYA library prep and run on a NovaSeq instrument. Differentially expressed (DE) genes between variables of interest (high vs. low ODI, high vs. low VAS, pairs of diagnoses, and duration of symptoms) were determined using the DESeq2 method with padj<0.05 considered statistically significant⁶.

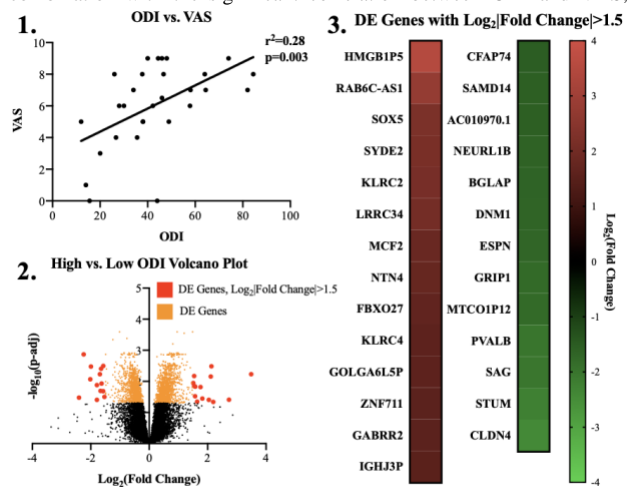
RESULTS: Duration of symptoms for all patients was 3 months or greater, indicative of a chronic condition. Few DE genes were identified in the comparisons of diagnosis, duration of symptoms, and VAS. However, a significant correlation was observed between ODI and VAS in this study population (Figure 1), and the comparison of high vs. low ODI resulted in 2,538 DE genes (Figure 2). Of the DE genes, 27 had a |log₂(Fold Change)| greater than 1.5, with 14 upregulated and 13 downregulated (Figure 3). Functionally, the top DE genes indicated that inflammation and the innate immune response (KLRC2, KLRC4, HMGB1P5, NTN4, GRIPI, NEURL1B) were implicated in the development of more severe disability. Transcriptomic indications of increased pain (NTN4, GABRR2) were also observed in patients with greater levels of disability.

DISCUSSION: ODI was found to be the variable yielding the greatest difference in the whole blood transcriptome of patients with chronic back pain. A significant correlation was observed between ODI and VAS, indicating that changes observed in the comparison of patient's ODI scores can be extrapolated as changes that also occur with VAS, or pain (Figure 1). It is possible that no DE genes were observed in the comparison of high vs. low VAS because of the subjectivity of the scoring system. The more extensive ODI questionnaire decreases subjectivity resulting in differential expression between high and low ODI (Figure 2). Within the top 27 DE genes, inflammation and the innate immune response were represented by 6 genes with KLRC2, KLRC4, HMGB1P5, and NTN4 upregulated and GRIPI and NEURL1B downregulated. KLRC2 and KLRC4 are known to be primarily expressed on natural killer (NK) cells⁷. Increased expression of these genes could be related to an increase in NK cell proliferation as well as inflammatory cytokines commonly produced by NK cells, including IFN γ ^{8,9}. Additionally, HMGB1P5, an inactive form of HMGB1, had the highest fold change of all DE genes and has been correlated with the functional form which is an inflammatory cytokine known to increase with increasing IVD degeneration severity^{4,10}. Immune cell infiltration into the IVD is also known to contribute to the progression of disease severity. Supporting this, the upregulation of NTN4 which plays a role in angiogenesis could increase potential for immune cell infiltration¹¹. Downregulation of GRIPI has also been shown to increase inflammatory mediator production, increase macrophage infiltration, and inhibit the transition in macrophage phenotype from pro-inflammatory M1 to anti-inflammatory M2¹². NEURL1B also modulates NOTCH signaling known to be involved in the M1 to M2 transition^{13,14}. The differential expression of these 6 genes supports the concept that increased disability levels are associated with an increased inflammatory and innate immune response. In combination with the significant correlation between ODI and VAS, transcriptomic changes indicative of increased pain were also observed in patients with greater levels of disability. Upregulated NTN4, mentioned above, has also been implicated in neurite ingrowth which is a common cause of pain in patients with spine pathology¹¹. Finally, GABRR2 was upregulated and is known to be involved in the modulation of pain originating in the spine (Figure 3)¹⁵. Taken together, these results support the notion that inflammation and the innate immune response contribute to the development of more severe disability and pain in patients with spine pathologies.

SIGNIFICANCE/CLINICAL RELEVANCE: Characterizing the transcriptomic changes in the whole blood of patients with spine pathologies could inform future treatment options that specifically target mechanisms known to lead to more severe disability and pain.

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1. Linear regression of ODI and VAS values for each patient. 2. Volcano Plot of DE genes between high and low ODI patients. 3. Heat map of DE genes with |Log₂(Fold Change)| greater than 1.5.