PRG4 Loss of Function Leads to Progressive Disc Degeneration

Daniel J. Garcia[1], Steven Muñoz[1], Gilbert Fermin[1], Zachary Boston[1], Sarah Folk[1], Marc Teitelbaum[1], Daniel Ortega[1], Abir Islam[1], David Paglia, PhD[1]
[1]Rutgers New Jersey Medical School, Newark, NJ, USA
Djg239@njms.rutgers.edu, smm578@njms.rutgers.edu, Fermin211@hotmail.com, zmb22@njms.rutgers.edu, smf296@njms.rutgers.edu, mht45@gsbs.rutgers.edu, dao2132@cumc.columbia.edu, abirislam96@gmail.com, pagliada@njms.rutgers.edu

Disclosures: Daniel J. Garcia (N), Steven M. Muñoz (N), Zachary Boston (N), Sarah Folk (N), Marc Teitelbaum (N), Daniel Ortega (N), Abir Islam (N), David N. Paglia (N)

INTRODUCTION: Disc degeneration is heavily correlated with back pain and is largely due to aging and the loss of proteoglycans and water[1]. One proteoglycan, lubricin (PRG4), functions in maintaining cartilaginous tissues by lubricating the boundary of articular surfaces to keep joint integrity. Lubricin deficiency is seen in osteoarthritis, degenerative joint disease, and Camptodactyly-arthropathy-coxa vara-pericarditis (CACP) syndrome. Several studies have demonstrated the spatial distribution of lubricin within the intervertebral disc[2,3] and provided evidence that loss of Prg4 signaling degrades the mechanical parameters of the disc, while reducing tissue volume[4]. This study postulates that the structure and distribution of collagen fibrils within the outer annulus and transition zone of the intervertebral disc (IVD) are governed by the spatial distribution of lubricin and elevated expression of lubricin in the nucleus pulposus correlates with degree of degeneration. We theorize Prg4 knockout may lead to differences in disc degeneration at different spinal levels.

METHODS: In this preliminary study, C57/Bl6 and Prg4 $^{-/-}$ mouse spines were harvested (at time points) for histology and PCR analysis. Intradiscal tissue was carefully dissected for PCR (n=3 per group), flash frozen, digested, and subsequently analyzed to evaluate how Prg4 loss of function affects genes associated with spine degeneration. For histologic analysis, spines from separate mice (n=6 per group) were fixed in Streck's Tissue fixative for 1 week, dehydrated through increasing concentrations of ethanol, decalcified with Ethylenediaminetetraacetic acid (EDTA), and embedded into paraffin wax until solid. Slides were prepared by cutting 6 micrometer sections using a microtome and then stained with Safranin O (for cartilage) and Fast Green (for cytoplasm/bone) (Figure 1). Discs were viewed with light microscopy and scored 0-12 based on 6 different parameters using a modified Thompson Scale. Score of each disc was statistically analyzed using Rank Sum Test. All C57/Bl6 control mice were housed under IACUC protocol #201800006. Prg4 null mice were housed under an approved IACUC at Rhode Island Hospital and non-study spinal tissue was donated by Dr. Gregory Jay, MD PhD. Immunohistochemical staining for lubricin protein expression in the disc was performed on slides from pristine and degenerated rabbit disc samples from a previous publication at UConn Health Center[5]. Permission to use slides for lubricin staining was granted by Dr. Issac Moss, MD, PI on rabbit PDGF study.

RESULTS: Histologic analysis of cervical intervertebral discs from Prg4 null mice had significantly higher average Thompson score compared to C57/Bl6 control mice indicative of more severe degeneration (7.56 vs 1.86, $p = 0.00235$). There were no significant differences in scores among thoracic sections between control and Prg4 null mice (6.25 vs 7.69, $p=0.725$). Lumbar intervertebral discs from Prg4 null mice had higher average scores compared to C57/Bl6 control mice (3.40 vs 8.74, $p = 0.0003$). Sacral intervertebral discs from Prg4 null mice also had higher average Thompson scores compared to the C57/Bl6 control mice (10.2 vs. 5.9, $p = 0.0485$). Within Prg4 null mice, Thompson scores in cervical vertebral discs were lower compared to those from lumbar discs, but this association was not significant (7.56 vs. 8.30, $p = 0.289$). PCR results suggest that Matrix metallopeptidase 9 (MMP9) has greater expression in Prg4 null discs, while Cox2 expression is inhibited in these samples. Immunohistochemical lubricin staining in rabbit discs shows expression in the outer annulus, transition zone (between annulus and nucleus), and adjacent to the growth plate in pristine control rabbits. In rabbits with mild disc degeneration, the primary lubricin protein expression is within the nucleus pulposus.

DISCUSSION: Through the modified Thompson scale, our results suggest how the loss of Prg4 expression due to mutation had reproducible effects on disc degeneration. Loss of Prg4 expression correlates with significant degeneration in both the sacral and cervical regions and increased gene expression of MMP9. Elevated MMP9 expression correlates with disc degeneration which supports the observations of this study. Induction of Prg4 by mechanical motion has been documented to be dependent on Cox2 signaling. The inhibition of Cox2 in PCR samples suggests a dysregulation of this signaling in mutant mice. When comparing discs from different regions within the same mouse, levels with sufficient samples (lumbar and cervical) were found not to be statistically significant. The histology observations in this study including disc collapse and multi-level disc fusion in mutant mice have not been previously reported. Limitations of the study include the absence of transcriptomic analyses to determine the signaling associated with the observed phenotypic changes and the lack of a rescue to ameliorate the phenotype of the mutation.

SIGNIFICANCE/CLINICAL RELEVANCE: Given the number of individuals who are affected by back pain and the costs that go into treating back pain, investigating Prg4 expression in intervertebral discs, locations where there are higher levels of lubricin expressed, and lubricin's effect on disc degeneration could elucidate new treatment modalities for alleviating cartilage degradation and back pain.

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Fig. 1: IVD Histology of C57/Bl6 control mice (Left) compared to Prg4 -/- mice (Right)