Nucleus Pulposus Runx1 Overexpression Accelerates Intervertebral Disc Aging in a Sex-dependent Manner

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Introduction: Intervertebral disc degeneration (IDD) is a major contributor to chronic back pain and disability worldwide[1]. Aging is a well-established risk factor for IDD. Emerging evidence in the literature points to sex-specific influence of IDD susceptibility and disease progression, indicating that male mice may exhibit more severe IDD progression than females during naturally aging[2]. The Acute Myeloid Leukemia factor Runx1 exerts pleiotropic effects including in mesenchymal tissues. We previously reported that Runx1 controls mesenchymal progenitor cell differentiation into the chondrogenic lineage[3] and that its expression is associated with nucleus pulposus (NP) degenerative tissue[4]. Our initial investigation revealed that nucleus pulposus (NP)-specific *Runx1* overexpression disrupts NP cell phenotype and increased hypertrophic matrix and senescence in male mice (5 to 10 months). Here, we investigated the consequences of Runx1 overexpression in the NP in young versus old male and female mice.

Methods: All procedures were performed with IACUC approval. Krt19-CreERT; Rosa26-Runx1$^{tg/+}$ mice were generated to induce NP-specific Runx1 overexpression following tamoxifen injection at 4 weeks. One week after tamoxifen injection, mouse NP tissues were collected and Runx1 expression was validated by western blot. Lumbar intervertebral discs (L3/4, L4/5, and L5/6) from both female and male mice were collected for histological analyses at 5 (young) and 14 (aged) months of age. Sections were stained with H&E to assess degeneration severity. Picrosirius Red/Alcian Blue staining was performed to evaluate ECM composition, focusing on proteoglycan versus collagen content in the NP. Quantification of histological and matrix staining was performed by blinded observers. Statistical comparisons were made using one way or two-way ANOVA with post hoc testing.

Results: Western blot confirmed significant Runx1 induction in the NP tissue of Krt19-CreERT;Rosa26-Runx1tg/+ mice compared with controls **(Figure 1).** Notably, females exhibited a greater fold increase in Runx1 protein expression than males, suggesting a sex-dependent regulation of Runx1 expression or stability in NP cells. Early degeneration at 5 months (data not shown): Histological scoring revealed that Runx1 overexpression mice of both sexes had significantly higher degeneration scores than controls at all lumbar levels examined. Degenerative features included reduced NP cellularity and altered NP morphology. A sex difference was observed specifically at L5–6, where females showed more severe degeneration than males in the discs of Runx1. L3–4 and L4–5 exhibited no sex difference at this early stage. Advanced degeneration at 14 months: By 14 months **(Figure 2),** mice with Runx1 overexpression displayed advanced disc degeneration characterized by NP matrix turnover and severe loss of cellularity. Histological scoring confirmed significantly greater degeneration in the discs of Runx1 overexpression mice compared to controls across all lumbar levels examined. Unlike the 5-month time point, sex differences were now evident at all levels, with females consistently showing higher degeneration scores than males. Matrix composition: Picrosirius Red/Alcian Blue staining revealed striking differences in ECM remodeling in the NP **(Figure 3).** Alcian Blue intensity, reflecting proteoglycan content, was reduced in Runx1 overexpression discs relative to controls but did not significantly differ between sexes. In contrast, Picrosirius Red staining, indicating collagen deposition (mainly collagen I and III), was markedly increased in the NP tissue of Runx1 overexpressing mice, with females exhibiting significantly higher collagen accumulation than males. In addition, the densely packed, mature lamellar collagen structure in the AF of control group became disorganized and immature in Runx1 overexpression group, as seen under polarized light. Again, the AF structure was more affected in females than males overexpressing Runx1.

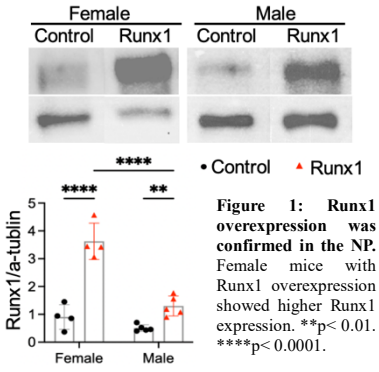

Figure 1: Runx1 overexpression was confirmed in the NP. Female mice with Runx1 overexpression showed higher Runx1 expression. **p< 0.01. ****p< 0.0001.

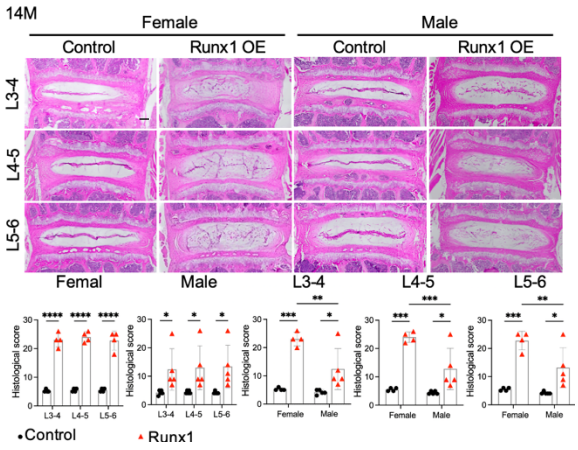

Figure 2: Runx1 overexpression in the NP accelerated disc aging. Female mice with Runx1 overexpression showed more degenerative changes than males in all levels examined. *p< 0.05. **p< 0.01. ***p< 0.001. ****p< 0.0001. Scale bar = 200um.

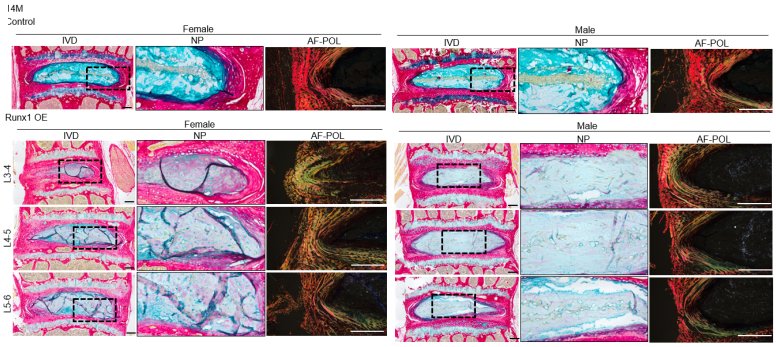

Figure 3: Runx1 overexpression induced more fibrotic matrix in the NP. Female mice with Runx1 overexpression showed more fiber collagen deposition in the NP than males in all levels examined. Scale bar = 200um.

Discussion: Our results build upon our recently completed study showing that Runx1 overexpression in male mice reduced notochordal marker expression and increased senescence markers at 5, 7, and 10 months. Here, we further showed sex-specific effects of Runx1 overexpression in age-related disc degeneration with accelerated aging of male and female discs overexpressing Runx1 in the NP compared to controls. The comparison between 5 and 14 months of age stems from the general appreciation that disc aging in C57Bl6 mice is apparent after 12 months of age. Early differences at L5–6 suggest regional susceptibility under Runx1 dysregulation, while long-term changes demonstrate a cumulative and sex-specific exacerbation of disc degeneration. Whether this sex-dependent response is mediated through Runx1 control of estrogen-responsive pathways[5] or other sex-dependent transcriptional regulators remains to be confirmed.

Significance/clinical relevance: These findings suggest that Runx1 is a driver of disc degeneration with sex-dependent effects. Targeting Runx1 mediated pathways may provide novel strategies to prevent or slow down disc aging and address sex-specific susceptibility to disc degeneration.

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