Assessing The Effect of Notochord-specific Deletion of Ccn2 on Intervertebral Disc Degeneration and Behavior Associated with Back Pain

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Introduction: Currently, our ability to treat intervertebral disc (IVD) degeneration is hampered by an incomplete understanding of the pathways that regulate disc aging as well as the factors contributing to symptomatic vs. asymptomatic disc degeneration. The specific function of matricellular proteins such as CCN2 (formerly known as connective tissue growth factor) and their ability to modulate these pathways remains enigmatic. Recent research by our group reported a mouse strain with notochord-specific deletion of CCN2 (targeting expression within the nucleus pulposus), and demonstrated the central role of this protein in regulating the extracellular matrix content of the IVD during both development and aging [1]. Building on these findings, herein we further elucidate the role of CCN2 during age-associated disc degeneration.

Methods: Intervertebral disc health was assessed in notochord-specific Ccn2-null mice and wild-type littermate controls at 6, 9, 13 and 18 months of age, using histological and immunohistochemical evaluation. In addition, we assessed functional behaviours associated with axial back pain by using grip force, tail suspension and locomotor assays to measure stretch-induced axial discomfort as previously described [2].

Results: Our previous studies showed that notochord-specific Ccn2-deficient mice show accelerated age-associated degeneration in both the nucleus pulposus (NP) and annulus fibrosus (AF) at 12 and 17 months of age, as determined by MRI analysis as well as histological grading of tissue appearance. To better characterize the onset of degenerative changes and the role of CCN2 in regulating IVD homeostasis, disc tissues were examined in mice at earlier time points. At 6 months of age, Ccn2-deficient mice showed no changes in tissue morphology compared to wild-type controls. Conversely, at 9 months of age mice lacking notochord-derived CCN2 show signs of early degeneration, such as the accumulation of fibrous tissue in the NP, resulting in significantly higher Thompson scores when compared to wild-type controls (P<0.001) (Figure 1). To determine whether alterations of IVD tissue structure in notochord-specific Ccn2-deficient mice are associated with increased activity of matrix remodelling enzymes, we assessed the abundance of matrix degradation fragments in the IVD. Although changes were not detected at either 6 or 9 months of age, immunolocalization showed a striking increase in the accumulation of MMP-mediated cleavage products in the NP of Ccn2-deficient mice at 18 months of age compared to wild-type controls. Lastly, behavioural assessment of mice at 13 and 18 months of age demonstrated a reduced ability to tolerate stretch-induced axial discomfort in Ccn2-deficient mice compared to wild-type controls. Ccn2-deficient mice show reduced resistive force during the grip force test relative to controls at both 13 (P<0.001) and 18 months of age (P<0.001). At 13 months of age, in the tail suspension test Ccn2-deficient mice show decreased time spent in full
extension (P<0.001) and an increased time spent self-supported (P<0.01), indicative of increased discomfort induced by gravitational axial stretching. At 13 months of age, the locomotor activity of Ccn2-deficient mice was also significantly reduced compared to wild-type controls (P<0.001) immediately following tail suspension, indicative of increased suspension-induced axial discomfort.

**Discussion:** Using a notochord-specific gene targeting strategy, we demonstrate that CCN2 expression by nucleus pulposus cells regulates IVD tissue health. Accelerated degeneration is initiated between 6 and 9 months of age in Ccn2-deficient mice, accompanied by increased MMP-mediated matrix degradation in the nucleus pulposus. Furthermore, our findings suggesting that Ccn2-null mice demonstrate symptomatic disc degeneration, providing valuable information regarding the utility of our mouse model given the disconnect between the manifestations of back pain and the radiographic appearance of disc degeneration in human patients.

**Significance:** The ability of CCN2 to regulate the composition of the intervertebral disc suggests that it may represent an intriguing target for the treatment of disc degeneration. Further, our Ccn2-deficient mouse model may be useful to probe the connection between disc degeneration and associated pain.

**Figure 1.** (A) Histological assessment of IVD tissue morphology by safranin-O/fast green staining. Ccn2-deficient mice show signs of early degeneration in the nucleus pulposus (NP) at 9 months of age that leads to further degeneration in both the NP and annulus fibrosus (AF) at 12 months of age. (B) Grading of tissue morphology using the Thompson grading scheme. Ccn2-deficient mice show significantly higher Thompson scores compared to wild-type controls, correlating to an increased severity of degeneration, in the NP at 9 months of age. *P<0.05, ***P<0.001. Scale bar represents 50 μm.

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