

Terminal Uridylyl Transferase 7 Deletion Suppressed Interleukin-6 Expression and Reduced The Severity Of Intervertebral Disc Degeneration In A Mouse Model

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Introduction. Aging and trauma are the primary risk factors linked with intervertebral disc (IVD) degeneration (IDD), which is a major cause of chronic neck, low back, and radicular pain in millions of people worldwide. Changes in the nucleus pulposus (NP) and annulus fibrosus (AF) cells are believed to trigger the loss of disc tissue by mechanisms that are poorly understood. Several studies have shown that increased interleukin-6 (Il-6) expression by IVD cells is an important contributor to IDD pathogenesis however the mechanism of Il-6 regulation in IVD is not fully explored. Terminal uridylyltransferase 7 (Tut7) is a zinc finger domain-containing protein with a nucleotidyl transferase domain that uridylates the 3' ends of specific miRNAs and mRNAs altering their stability and function. Here, we explored the role of Tut7 in the regulation of Il-6 expression in trauma and aging-induced IDD using a global Tut7KO mouse model.

Methods. All the studies using the animal model were reviewed and approved by NEOMED IACUC. Both male and female aged (up to 15 months) wt and Tut7KO mice were included in this study to investigate aging-related changes in lumbar and caudal IVDs. Needle puncture surgery (NPS) was done on 10-12 weeks-old mouse caudal discs (ca4/5 and ca6/7) to mimic trauma-induced IDD. The mice were euthanized at 2 weeks post NPS and the tail was harvested and processed for microCT to determine changes in bone and disc height and histology to determine disease severity. For histological evaluation of IDD, the lumbar or tail samples were collected and fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, and cut into 5 μ m thin sections and stained with H&E, Toluidine blue or Picrosirius red. The expression of Tut7, Il-6, nociceptor neuronal markers (SP and CGRP), and matrix-degrading proteases in the IVDs was determined by immunostaining. Gene expression in IVDs was determined by RT-qPCR by isolating total RNA from the lumbar and caudal discs of wt and Tut7KO male and female mice (n=6 per age group). Primary mouse NP and AF cells were prepared by sequential enzymatic digestion of the NP and AF tissue from lumbar and caudal discs with Proanse and Collagenase. Primary NP and AF cells were treated with recombinant mouse Il-1 β (5 ng/ml) and the cells were harvested for RNA isolation, followed by RT-qPCR for gene expression analyses. The MERCY scoring system was used to determine the severity of the IDD. The cell count and area of NP in the lumbar and caudal discs (c3-c9) of wt and Tut7KO were measured using ImageJ software. The human NP cells were treated with Il-1 β (5 ng/ml) for up to 48 h and total RNA was isolated to examine the expression of Tut7. The data were analyzed by unpaired, two-tailed students t-test or one-way ANOVA for statistical significance.

Results. Tut7 expression was higher in aged (15-month-old) and punctured IVDs of wt C57BL6 mice and correlated with increased levels of Il-6. Tut7 deletion in mice significantly reduced the severity of age-related IDD in both the lumbar and caudal regions compared to age and sex matched wt littermates. Compared to wt, Tut7KO mice also showed reduced IDD in the NPS model. Interestingly, NPS Tut7KO IVDs expressed low levels of Il-6, and other catabolic markers compared to wt NPS IVD. In addition, SP and CGRP-positive nociceptor nerve fibers were low in Tut7KO NPS IVD indicating reduced disc hyperalgesia. Tut7KO-aged IVDs also expressed low levels of Il-6 in both NP and AF compartments compared to age and sex-matched wt littermates. Tut7KO also expressed low levels of Mmp13 and Cox-2 suggesting reduced Il-6 expression inhibits downstream catabolic genes. Tut7 deletion prevented the aging and trauma-induced loss of NP cells in mouse lumbar and caudal IVDs. The expression of senescence marker, p16^{ink4a}, and matrix-degrading proteases, Mmp13 and Adamts5 was also low in aged (15 months) Tut7KO mouse IVDs in comparison to age and sex-matched wt littermates. Interestingly we found increased levels of matrix synthesis genes, type II collagen and aggrecan in aged Tut7KO mice IVDs. Treatment of wt mouse AF cells with Il-1 β (5 ng/ml) significantly increased the expression of Tut7, p16^{ink4a}, Il-6, Adamts5, and Mmp13 as determined by RT-qPCR showing a direct relationship between Tut7 and Il-6 expression. In addition, IL-1 β (5 ng/ml) treatment also increased TUT7 expression in primary human NP cells.

Discussion. This study shows that Tut7 promotes disc degeneration by increasing the expression levels of Il-6 in IVDs. In this study, we showed that Tut7 deletion in a mouse model of aging and trauma-induced IDD suppressed Il-6 expression, matrix degradation, pain sensitivity and increased the expression of extracellular matrix genes, and significantly reduced the severity of age-related and trauma-induced IDD. Our data identifies Tut7 as a critical regulator of inflammation in IVDs.

Significance. We demonstrated here that Tut7 plays a critical role in IDD by promoting inflammation. This preclinical study identifies Tut7 as a potential therapeutic target for the management of IDD.

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