

Discoidin domain receptor 1 (DDR1) negatively regulates intervertebral disk degeneration

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INTRODUCTION: Intervertebral disc degeneration (IDD) constitutes a significant etiological factor for low back pain (LBP). IDD is characterized by the depletion of resident cells and degradation of the extracellular matrix (ECM). The increased senescence and apoptosis of nucleus pulposus (NP) cells may contribute to excessive cell death, thereby hastening IDD progression. Discoidin domain receptor 1 (DDR1) is a transmembrane receptor that binds to collagens. In our previous studies, we generated inducible type II collagen (Col-2)-specific Ddr1 knockout mice (CKOΔDdr1 mice), wherein cells expressing Col-2 in the cartilaginous endplate (CEP), annulus fibrosus (AF), and NP of the intervertebral disc (IVD) do not express DDR1. Additionally, we confirmed that DDR1 is essential for postnatal intervertebral disc growth and development through the FoxA-Shh-Gli1/2-Pax1-SOX9 pathway. These findings highlight the potential significance of DDR1 in physiology of IVD. Therefore, our study aims to investigate the role of DDR1 in IDD, hypothesizing that DDR1 likely plays a pivotal role in regulating IDD.

METHODS: The Ddr1^{fl/fl} mice were as the control group. The CKOΔDdr1 mice are the treatment group that were injected 4-hydroxy-tamoxifen(4-OHT) to induce DDR1 knockout. The Ddr1^{fl/fl} mice and CKOΔDdr1 mice with 4-OHT injection from 2-month-old or 4-month-old are used and they were sacrificed at 6 to 12-month-old. The microstructures of IVD were evaluated by GAG staining and the apoptotic cells were evaluated by TUNEL staining, respectively. IHC staining for type II collagen and senescence marker, p16, were also evaluated in control and CKOΔDdr1 mice. To further elucidate DDR1's role in intervertebral disc degeneration (IDD), the DDR1 inhibitor 7-rh was used to block DDR1 function. Additionally, IL-1 was utilized to mimic degenerative conditions in human nucleus pulposus (NP) cells for *in vitro* studies.

RESULTS: Our results demonstrated a decrease in DDR1 expression with age in the intervertebral disc (IVD) tissues of normal mice. However, in CKOΔDdr1 mice, the expression of DDR1 was significantly reduced, indicating a persistent effect of the knockout (Fig 1A). Safranin O staining and IHC staining were employed to assess GAG composition, IVD microstructure, and COL-2 expression levels (Fig 1B). The results revealed that CKOΔDdr1 mice exhibited larger IVD structures with higher levels of collagen 2 in the NP region. Upon further examination of cell apoptosis and p16 expression, it was observed that the IVD of CKOΔDdr1 mice contained a higher number of apoptotic cells (Fig 2A) and fewer p16-positive cells (Fig 2B). Furthermore, when human NP cells were exposed to IL-1β and subsequently treated with the DDR1 inhibitor 7-rh *in vitro*, it was observed that the expression of DDR1 gene decreased after IL-1 treatment, but no significant change after 7-rh treatment (Fig 3A). Moreover, IL-1 and COL-X gene expression increased following IL-1 treatment and they can be reduced through treatment with 7-rh (Fig 3B and C). Conversely, the gene expression of SOX-9 were reduced in IL-1 treatment, but remained unchanged after 7-rh treatments (Fig 3D).

DISCUSSION: Base on our *in vivo* results, CKOΔDdr1 mice exhibited larger IVD structures with higher levels of collagen 2 in the NP region. It inferred that the knockout of DDR1 leads to reduced IVD degradation and improved structural preservation of the IVD. The NP cells contain two distinct cell types: large clusters of notochordal cells (NC cells) and small chondrocyte-like cells. The NP cells encompass two types: notochordal cells (NC) and small chondrocyte-like cells. Recent research highlights chondrocyte-like cells deriving from degenerating NP cells, gradually replacing the original notochordal cells. Further examination showed CKOΔDdr1 mice's IVD had more apoptotic cells and fewer p16-positive cells, implying increased cell turnover and reduced senescent cells. We will further investigate the composition and functions of NC cells and chondrocyte-like cells in Ddr1^{fl/fl} mice and CKOΔDdr1 mice. Furthermore, in *in vitro* study, IL-1 treatment increased cellular stress in hNP cells, and the gene expression of IL-1 and COL-X can be reduced through treatment with 7-rh. However, the expression of SOX-9 remained unchanged while inhibition of DDR-1 didn't improve the chondrogenic function but reduce the inflammatory response. By integrating the results, it becomes evident that inhibiting DDR1 has the potential to delay degeneration and reduce cellular senescence in the process of intervertebral disc degeneration in mice. This effect links to inflammaging modulation and DDR1's negative role in IVD degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: This finding not only enhances our comprehension of the plausible mechanisms underlying intervertebral disc degeneration (IDD) but also provides the way for innovative therapeutic avenues, leveraging DDR1 inhibition as a potential approach.

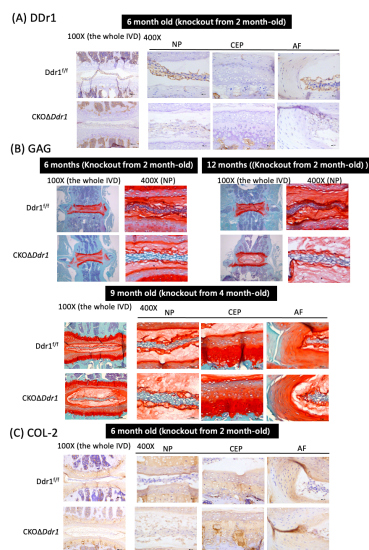


Fig 1. (A) The DDR1 expression in Ddr1^{fl/fl} mice and CKOΔDdr1 mice. (B) The microstructure of IVD in Ddr1^{fl/fl} mice and CKOΔDdr1 mice at 6- to 12-month-old. (C) The collagen 2 expression in Ddr1^{fl/fl} mice and CKOΔDdr1 mice.

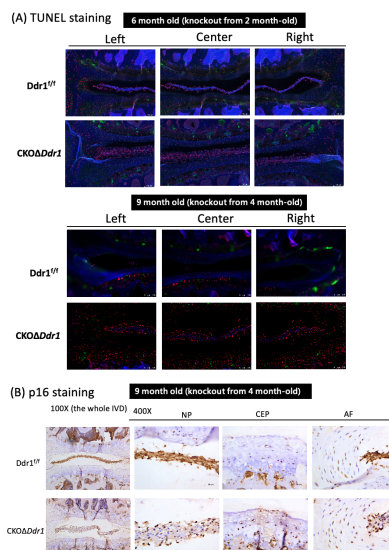


Fig 2. The TUNEL staining and IHC for p16 (A) The IVD of CKOΔDdr1 mice contained a higher number of apoptotic cells. (B) The p16 IHC staining remained more positive cells in Ddr1^{fl/fl} mice than in CKOΔDdr1 mice

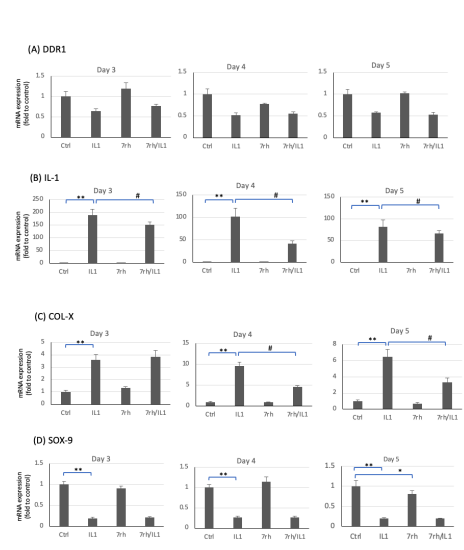


Fig 3. The gene expression in hNP cells after IL-1 and 7-rh treatment. (A) DDR1 (B) IL-1 (C) COL-X (D) SOX-9. (*, p<0.05, **, p<0.01 compared with control group; #, p<0.05 compared with IL-1 alone group)