DDR1-Mediated Regulation of Inflammaging in Intervertebral Disc Degeneration

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INTRODUCTION: Intervertebral disc degeneration (IDD) is a primary cause of lower back pain (LBP). IDD is characterized by the depletion of resident cells and the degradation of the extracellular matrix (ECM). Apoptosis, senescence, autophagy, and mitochondrial may damage in nucleus pulposus (NP) cells lead to excessive cell death, accelerating the progression of IDD. Our previous studies have shown that DDR1 plays a crucial role in development, suggesting it may also regulate IDD. Therefore, our study aims to investigate the role of DDR1 in IDD, hypothesizing that DDR1 likely plays a role in regulating IDD.

METHODS: The $Ddr1^{f/f}$ 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 $Ddr1^{f/f}$ mice and CKOΔDdr1 mice with 4-OHT injection from 4-month-old are used and they were sacrificed at 9-month-old. The microstructures of IVD were evaluated by GAG staining. IHC staining for IL-1, MMP-13 and AGE (advanced glycation end products) and senescence marker, p16, p53 and p21 were also evaluated in control and CKOΔDdr1 mice. To further elucidate DDR1's role in intervertebral disc degeneration (IDD), the DDR1 inhibitor 7-rh or siRNA was used to block DDR1 function in human nucleus pulposus (NP) cells for *in vitro* studies. Additionally, IL-1 was utilized to mimic degenerative conditions. The mRNA expression and protein levels were evaluated. Moreover, we also did mRNA NGS analysis to evaluate the GO and KEGG function.

RESULTS: Our results demonstrated a decrease in DDR1 expression with age in the intervertebral disc (IVD) tissues of normal mice (data not showed). 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 for type 2 collagen revealed that CKOΔDdr1 mice exhibited larger IVD structures with GAG and type 2 collagen (data not showed). Upon further examination of cell aging markers, p16, p53 and p21 expression, it was observed that the IVD of CKOΔDdr1 mice contained fewer positive cells (Fig 1C-E). The IHC staining of inflammation and degenerative marker such as IL-1, MMP-13 and AGE also showed the IVD of CKOΔDdr1 mice contained fewer positive cells. Furthermore, when human NP cells were exposed to IL-1β and subsequently treated with the DDR1 inhibitor 7-rh or siRNA DDR1 *in vitro*, it was observed that the expression of DDR1gene decreased after IL-1 treatment, but no significant change after 7-rh treatment (Fig 3A). Moreover, IL-1, MMP-13 and COL-X gene expression increased following IL-1 treatment, and they can be reduced through treatment with 7-rh (Fig 3A). The protein levels of IL-1, p16, p53 and p21 were increased after IL-1 treatment and reversed by siRNA DDR1 treatment (Fig. 3B). GO and KEGG analyses indicated that IL-1 treatment may promote inflammation and cellular aging by enhancing ROS production and impairing mitochondrial function, whereas DDR1 inhibition may attenuate inflammaging (Fig 3C). It showed that DDR1-Mediated regulation of inflammaging in IDD.

DISCUSSION: Based 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 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 fewer aging 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 $Ddr1^{f/f}$ 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, MMP-13 and COL-X can be reduced through treatment with 7-rh. The protein levels of p16, p53 and p21 showed silencing DDR1 may reduce the aging markers. 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. According to our NGS analysis, these effects links to ROS production and mitochondrial function. Our study showed that DDR1 may modulate inflammaging which related to ROS and mitochondrial function in IVD degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings elucidate potential mechanisms of intervertebral disc degeneration (IDD) and highlight DDR1 inhibition as a promising therapeutic strategy.

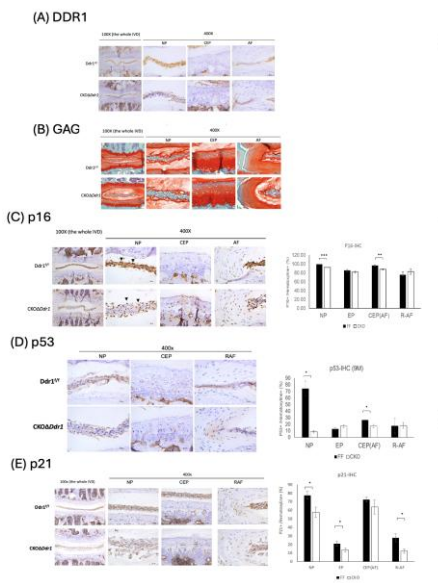

Fig 1. (A) The DDR1 expression in $Ddr1^{f/f}$ mice and CKOΔDdr1 mice. (B) The microstructure of IVD in $Ddr1^{f/f}$ mice and CKOΔDdr1 mice (C-E) The p16, p53 and p21 expression in $Ddr1^{f/f}$ mice and CKOΔDdr1 mice.

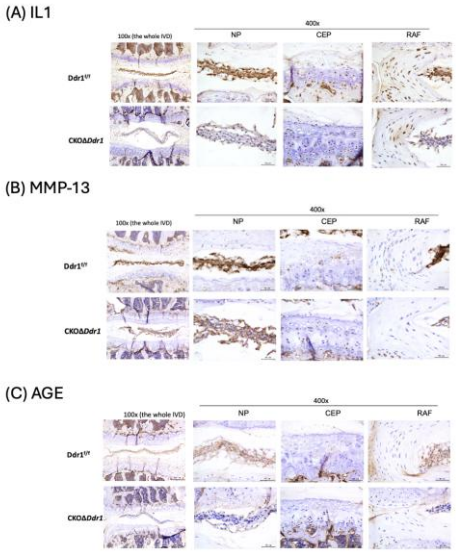

Fig 2. The IHC staining of IVD in $Ddr1^{f/f}$ mice and CKOΔDdr1 mice. (A) IL-1 (B) MMP-13 (C) AGE.

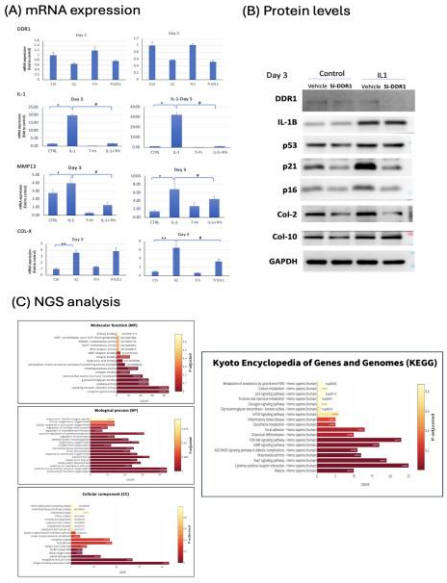

Fig 3. (A) The gene expression in hNP cells after IL-1 and 7-rh trearment. (B) The protein levels of hNP cells after siRNA DDR1 treatment (C) The GO and KEGG analysis in hNP cells by NGS analysis. (*, $p<0.05$, **, $p<0.01$ compared with control group; #, $p<0.05$ compared with IL-1 alone group)