Progranulin Deficiency Causes Intervertebral Disc Degeneration in Aging Mice

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Introduction: Progranulin (PGRN) is a multifunctional growth factor which plays a critical role in various physiological and disease processes. Recently, we reported in Science that PGRN directly bound to TNF receptors (TNFR), blocked the binding of TNF to TNFR and inhibited TNF activity in inflammatory arthritis and bone regeneration. The study aimed examining PGRN expression in intervertebral disc (IVD) under physiological and pathological degenerative conditions, defining the role of PGRN in IVD degeneration in aging, and elucidating the signaling pathways involved.

Methods: The samples of IVD came from murine subjects. The degeneration of the IVD samples were analyzed by HE staining, Safranin O staining, immunohistochemistry and µCT. The expressions of genes associated with cartilage degeneration, osteoblastogenesis and osteoclastogenesis were analyzed. We also analyzed the IVD samples from wildtype and PGRN-/- mice for the NF-κB signaling pathways.

Results: PGRN is expressed in both human and murine IVD tissue and PGRN level is elevated in murine IVD during aging.

To investigate the potential involvement of PGRN in disc degeneration of human being, we examined its expression pattern in IVD tissue disc degeneration patients. Immunohistochemistry results demonstrated PGRN was detectable in cell clusters formed in NP (Figure 1A, upper panel) and AF (Figure 1B, upper panel). High-resolution analysis found that in the cell clusters formed in NP (Figure 1A, lower panel) and AF (Figure 1B, lower panel). To determine the expression pattern of PGRN in mice IVD, we performed immunohistochemistry with both 2- and 9-month old wild type mice, and found that PGRN was detectable in the EP and AF region but not NP (Figure 1C and 1D).

Deficiency of PGRN accelerates new bone formation and destruction of cartilaginous structure in IVD
In 6-month old mice, new bone formation in IVD tissue was detected via micro CT and histology (Figure 2A), while loss of proteoglycan was much more severe in the endplate cartilage of PGRN deficiency mice, accompanied by newly formed bone, and high-resolution analysis showed that cell clusters were formed in EP (Figure 2B). As shown in Fig 2C, TRAP staining of 6-month old spine showed remarkably elevated osteoclast activity within both the newly formed bone tissue (in EP cartilage) and the trabecular bone in PGRN-/- mice, which was not present in the WT littermates. To verify the osteoclast activity in spine, we used micro CT to analyze the trabecular bone in L4 vertebra of 6-month old WT and PGRN-/- mice, and found that PGRN deficient mice exhibited lower quality of trabecular bone.

PGRN deficiency leads to augmented NF-κB and Wnt/β-catenin signaling in IVD
To further determine the effects of PGRN deficiency on the activation of NF-κB signaling, immunohistochemistry was performed for phosphorylation of IκBα, and 6-month old PGRN-/- mice demonstrated remarkably higher signal of pIκB-α around nuclei of cells in EP compared with WT controls (Figure 3A). As shown in Figure 3B, total IVD extracts were collected from both WT and PGRN-/- mice and western blotting was performed. As shown in Figure 3C, β-catenin signal was stronger and the nuclear translocation of β-catenin was observed in IVD tissue of PGRN-/- mice by immunohistochemistry staining. To further investigate the activity of Wnt/β-catenin signaling, expression level of downstream
target genes including Axin2 and RUNX2 in IVD of 6-month old WT and PGRN-/- mice were measured through real time RT-PCR. Figure 3D determined that Axin2 level and RUNX2 level was significantly higher in PGRN-/- IVD, suggesting the activation of Wnt/β-catenin signaling pathway. Collectively, PGRN deficiency can causes intervertebral disc degeneration via NF-κB and Wnt/β-catenin signaling pathway(Figure 3G).

**Discussion:** PGRN plays a critical role in homeostasis of IVD through interacting with vertebra bone and intervertebral disc. In addition, PGRN protects against the intervertebral disc degeneration by inhibiting NF-κB and Wnt/β-catenin signaling pathway.

**Significance:** Our group first identify that PGRN plays a critical role in homeostasis of IVD, and the deficiency of PGRN leads to an acceleration in disc degeneration with aging. These findings show that PGRN is a potential molecular target for prevention and treatment of disc degenerative diseases.

![Image of PGRN expression in IVD tissues](image.png)

*Fig. 1. PGRN is expressed in disc tissues of both humans and mice and its level is elevated in mice IVD through aging. (A) PGRN was detectable in the extracellular matrix of the cell clusters in normal NP (upper panel) and AF (lower panel) from degenerated discs. Samples from disc degeneration patients (n=7) were collected and were stained with anti-PGRN antibody (brown), then counterstained with methyl green (green). Representative pictures are shown. The insets indicate higher magnification views of cell clusters. Scale bar: 25μm. (B) and (C) Signal of PGRN in IVDs from 2-month and 9-month-old mice, detected by immunohistochemistry. Positive staining was detected particularly in NP and AF of IVD. Representative pictures are shown and red arrows indicate PGRN expression in IVD. Scale bar: 100μm.*
Fig 2 Deficiency of PGRN accelerates bone formation and destruction of cartilaginous structure in IVD
(A) New bone formation in EP of 6-month old PGRN/- mice (red arrows). IVD samples from 6-month old WT and PGRN/- mice were collected then microCT and HE staining were performed.
(B) 6-month old PGRN/- mice revealed formation of cell clusters (blue arrows) and new bone (yellow arrows) in IVD, assayed by Safranin O staining.
(C) Higher activity of osteoclast in IVD and adjacent vertebra of 6-month old PGRN/- mice (black arrows), determined by TRAP staining and osteoporosis change in trabecular bone of L4 vertebra in 6-month old PGRN/- mice, assayed by microCT.
Fig 3 PGRN deficiency leads to augmented NF-κB and Wnt/β-catenin signaling pathway in IVD
(A) Enhanced phosphorylated IkB-α (pIKB-α) signaling in cytoplasm of EP cells (black arrows), tested by immunohistochemistry (B) Increased expression of pIKB-α in IVD, assayed by Western Blotting. Total protein extracts were collected from 3 mice of each group and Western Blotting was performed. (C) PGRN-/− mice revealed stronger β-catenin signal (middle) and enhanced nuclear translocation (right) in IVD cells (black arrows), determined by immunohistochemistry. (D) Elevated mRNA levels of AXIN2, RUNX2 in IVD of PGRN-/− mice, measured by real-time PCR. RNA was collected from IVD of 6-month WT and PGRN-/− mice, followed by real-time PCR (E) A proposed model for explaining the protective role of PGRN in the course of IVD degeneration

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