Progranulin Protects Against Osteoarthritis Through Interacting With Tnf-α And β-catenin Signaling Pathway

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Introduction: Progranulin (PGRN) was previously isolated as an osteoarthritis (OA)-associated growth factor. Additionally, PGRN was found to play a therapeutic role in inflammatory arthritis mice models through antagonizing TNF-α (Tang, et al, Science, 2011). This study was aimed at investigating the role of PGRN in degradation of cartilage and progression of OA.

Methods: Progression of OA was analyzed in surgically induced OA models in wild type and PGRN-deficient mice. Cartilage degradation and OA were evaluated using Safranin O staining and ELISA assay. Additionally, mRNA expression of degenerative factors and catabolic markers known to be involved in cartilage degeneration in OA were analyzed. Furthermore, the anabolic effects and underlying mechanisms of PGRN were investigated by in vitro experiments with primary chondrocytes.

Results: Deficiency of PGRN leads to exaggerated OA in surgically induced OA models
To study the role of PGRN in vivo, a surgically induced DMM OA model was established in both WT (n=7) and PGRN−/− (n=7) mice. Safranin O staining (Figure 1A) revealed that more severe structure loss was presented in PGRN−/− mice than in WT mice. OARSI scoring of OA was performed, and Figure 1B demonstrated that PGRN−/− mice had a significantly higher arthritic score than the WT group at all the time points. In addition, sera were collected from both genotypes at indicated time points following DMM operation and assayed through COMP fragment-specific ELISA. The result revealed that deficiency of PGRN led to enhanced degradation of COMP after DMM operation (Figure 1C).

Recombinant PGRN protects against OA through inhibiting TNF-α signaling pathway
Cartilage samples from patients with OA were isolated and cultured with 10 ng/mL TNF-α in presence or absence of 200 ng/mL PGRN for 48 h. As revealed in Figure 2A and 2B, Safranin O staining of the cartilage samples indicated that TNF-α markedly enhanced loss of proteoglycan, while the additional use of PGRN largely rescued this effect of TNF-α. To investigate whether the anabolic effects of PGRN on chondrocytes depend on TNFRs. Articular chondrocytes from new-born WT, TNFR1−/− and TNFR2−/− mice were isolated and cultured for 1 week until confluent. Then the chondrocytes were treated with or without 200 ng/mL PGRN for 48 hours before real-time PCR was performed. As shown in Figure 2C and 2D, PGRN significantly elevated the levels of Aggrecan and Col II in WT, TNFR1−/− and TNFR2−/− chondrocytes, while the levels of anabolic factors were significantly higher in PGRN-treated WT and TNFR1−/− chondrocytes than in TNFR2−/− chondrocytes, which may indicate the anabolic effect of PGRN was significantly diminished when TNFR2 was deleted.

β-Catenin signaling is also involved in the PGRN-mediated protection of OA
Immunohistochemistry of β-catenin was performed in cartilage of 6-month-old WT and PGRN−/− mice. As shown in Figure 3A, β-catenin signal was stronger in cartilage tissue of PGRN−/− mice. Furthermore, cartilages from 6-month-old WT and PGRN−/− mice were harvested, and total RNA was extracted for real-time PCR assay. As shown in Figure 3B, mRNA level of β-catenin was significantly higher in cartilage
of PGRN−/− group. To further investigate Wnt/β-catenin signaling, expressions of downstream target genes, including Axin2 and RUNX2, in cartilage of 6-month-old WT and PGRN−/− mice were measured through real-time PCR. Axin2 and RUNX2 were both significantly upregulated in PGRN−/− articular cartilage (Figure 3C, D). To further elucidate the importance of β-catenin signaling in mediating PGRN activity in chondrocytes and OA, primary chondrocytes were isolated from 6-month-old WT and PGRN−/− mice, and the effect of β-catenin inhibitor FH535 was examined. As indicated in Figure 3E and 3F, PGRN−/− chondrocyte exhibited significantly higher levels of RUNX2 and Axin2, while this elevation was largely abolished with the addition of β-catenin inhibitor. Additionally, β-catenin-specific reporter gene assay was also measured. PGRN deficiency led to increased luciferase activity (Figure 3G). Collectively, these data indicated that PGRN-activated signaling and anabolic metabolism in chondrocytes is primarily mediated through both TNFR and β-catenin pathways (Figure 4).

**Discussion:** NA

**Significance:**

Our group provides new insight into the pathogenesis of OA, and also presents PGRN as a potential target for the treatment of joint degenerative diseases, including OA.
Fig 1 Deficiency of PGRN leads to exaggerated OA phenotype in surgically induced arthritis models

(A) PGRN−/− mice exhibited accelerated degeneration of articular cartilage compared with wild type (WT) littermates, assayed by Safranin O staining. Red arrows indicated cartilage destruction. Scale bar=100 μm.

(B) OARSI score of osteoarthritis based on the Safranin O staining.

(C) PGRN−/− mice presented a significantly higher level of COMP fragment in serum after induction of DMM model, assayed by ELISA.
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Fig 2 Recombinant PGRN protects against OA through inhibiting TNF-α signaling pathway
(A) PGRN significantly attenuated loss of proteoglycan in cartilage induced by TNF-α, assayed by Safranin O staining. Cartilage samples were isolated from patients with OA and cultured with 10 ng/ml TNF-α, in presence or absence of 200 ng/ml PGRN for 7 days.
(B) Statistic analysis based on the Safranin O staining.
(C), (D) mRNA levels of collagen 2 and Aggrecan. Primary chondrocytes were isolated from newborn wild type (WT), TNFRI1-/- and TNFRI2-/- mice, and cultured in presence or absence of 200 ng/ml PGRN for 48 h, followed by collection of total RNA and real-time PCR assay.

Fig 3 Recombinant PGRN protects against OA through β-catenin signaling pathway
(A) Detection of β-catenin in cartilage of 6-month-old wild type (WT) and PGRN-/- mice, assayed by immunohistochemistry. Scale bar=100 μm. (B)-(D) Levels of β-catenin and its downstream molecules including RUNX2 and Axin2 in cartilage of 6-month-old WT and PGRN-/- mice, assayed by real-time PCR. (E) and (F) Levels of RUNX2 and Axin2 in chondrocyte of 6-month-old WT and PGRN-/- mice, in the presence or absence of β-catenin inhibitor, as measured by real-time PCR. (G) Additional treatment of PGRN improved exaggeration of β-catenin level in PGRN-/- chondrocyte, as detected by luciferase reporter gene assay. (H) A proposed model for the role of PGRN in osteoarthrisis development.

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