Lysosomal-mediated Regulation of Chondrocyte Metabolism via the Linking of PGRN and GBA Mutation

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Disclosures: None

Introduction: Lysosomes are the primary catabolic organelles responsible for degrading cellular waste and damaged components, thereby regulating organelle turnover and cell death. Age-related loss of lysosomal function has been linked to mitochondrial dysfunction and chondrocyte apoptosis. Osteoarthritis (OA), a degenerative joint disease, is strongly associated with aging. Progranulin (PGRN, encoded by Grn) is a lysosomal protein, expressed in diverse cell types, with critical roles in physiological and pathological processes. Elevated PGRN levels have been observed in the cartilage of patients with OA and rheumatoid arthritis (RA), while PGRN deficiency aggravates surgically induced OA phenotypes. Our previous studies identified PGRN as a novel regulator of glucocerebrosidase (GCase), a lysosomal enzyme encoded by GBA1 that is essential for sphingolipid metabolism [1]. Moreover, PGRN deficiency exacerbates lysosomal storage disease phenotypes associated with GBA1 mutations [2]. The objective of this study is to investigate how lysosomal dysfunction regulates chondrocyte metabolism through the interplay between PGRN and GBA1 mutations.

Methods: Conduritol B epoxide (CBE) was used to inhibit GCase activity in control and PGRN-knockout C28/I2 chondrocytes. GCase activity was measured using the 4-Methylumbelliferone (MUB), while lysosomal dysfunction was assessed by LysoTracker Red staining, to evaluate the synergistic effect of PGRN and GBA1 deficiency on chondrocyte lysosomal function. To generate PGRN and Gba1 double-mutant mice, $Grn^{-/-}$ mice were crossed with $Gba1^{D409V/D409V}$ mice (encoding mutant GCase), producing $Grn^{-/-}Gba1^{D409V/D409V}$ ($Grn^{-/-}Gba1^{9V/9V}$) mice. The synergistic effect of PGRN and Gba1 deficiency on osteopenia and chondrocyte metabolism was analyzed in 12-month-old mice using micro-CT and immunohistochemical staining.

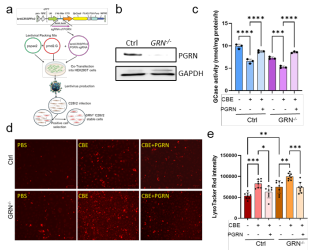

Figure 1. GRN deletion further impairs lysosomal function in human chondrocytes with deficient GCase activity. (a) Workflow of generating GRN knockout C28/I2 cells using CRISPR/Cas9 technology. (b) Western blot confirmation of GRN KO in C28/I2 cells. (c) GCase activity in control and GRN KO C28/I2 cells with or without CBE or rPGRN treatment assayed by release of 4-methylumbelliferone. (d) (e) Lysosome activity in control and GRN knockout C28/I2 cells with or without CBE or rPGRN treatment through LysoTracker Red staining.

Results: Loss of GRN exacerbates lysosomal dysfunction in human chondrocytes with reduced GCase activity. Given that PGRN is a lysosomal protein and a novel regulator of the lysosomal enzyme GCase, we investigated lysosome-regulated chondrocyte metabolism through the PGRN–GCase link. PGRN was deleted in C28/I2 chondrocytes using CRISPR/Cas9 technology (**Fig. 1a,b**). To inhibit GCase activity and impair lysosomal function, cells were treated with CBE. As shown in **Fig. 1c**, GCase activity was reduced following CBE treatment and was further decreased in PGRN KO C28/I2 cells compared with controls. LysoTracker staining revealed a significant increase in lysosomal content in PGRN KO cells relative to controls after CBE treatment (**Fig. 1d,e**).

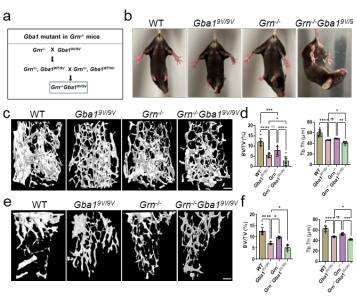

Figure 2. PGRN deficiency induced behavioral deficits and bone loss in 12m-old Gba1 mutant mice. (a) Schematic of the mouse breeding strategy to generate $Grn^{-/-}Gba1^{9V/9V}$ mice through crossing $Grn^{-/-}$ mice with $Gba1^{9V/9V}$ mice. (b) 12m-old $Grn^{-/-}Gba1^{9V/9V}$ mice showed hind limb clasping in comparison with WT, $Gba1^{9V/9V}$, and $Grn^{-/-}$ mice displayed no clasping. Representative reconstructed 3D micro-CT images of femur (c) and tibia (e) trabecular bone of WT, $Gba1^{9V/9V}$, $Grn^{-/-}$ and $Grn^{-/-}Gba1^{9V/9V}$ mice. Quantification of BV/TV and Tb. Th of femur (d) and tibia (f) trabecular bone. (n = 3 mice for each group). Scale bar = 250 μm.

Loss of PGRN exacerbated behavioral impairments and bone loss in 12m-old Gba1 mutant mice. Since OA is an age-related degenerative disease, we next deleted PGRN in Gba1 mutant mice to examine the effects of PGRN deficiency on bone loss and osteopenia in the context of Gba1 mutation induced lysosomal dysfunction (**Fig. 2a**). At 12 months of age, $Grn^{-/-}Gba1^{9V/9V}$ mice exhibited severe behavioral deficits, including abnormal hindlimb clasping, which was not observed in age-matched control groups (**Fig. 2b**). To assess the effects of PGRN on osteopenia, long bones (femur and tibia) and knee joints were collected from 12m-old WT, $Gba1^{9V/9V}$, $Grn^{-/-}$, and $Grn^{-/-}Gba1^{9V/9V}$ mice. Following fixation in 4% paraformaldehyde, samples were analyzed by micro-CT imaging. Bone volume/tissue volume (BV/TV, %) and trabecular thickness (Tb.Th) of the femur and tibia were quantified using 3D analysis with CT-Analyser (Bruker). Micro-CT analysis revealed that PGRN deficiency significantly reduced bone mass in the long bones, with decreases in BV/TV and Tb.Th in both femur (**Fig. 2c,d**) and tibia (**Fig. 2e,f**), compared with age-matched WT, $Gba1^{9V/9V}$, and $Grn^{-/-}$ mice.

The synergistic effect of PGRN and Gba1 exacerbated cartilage degeneration in aged mice. We next investigated whether PGRN deficiency exacerbates cartilage degeneration in the context of Gba1 mutation induced lysosomal dysfunction. Following micro-CT imaging, knee joints were decalcified in 10% (w/v) EDTA for three weeks, embedded in paraffin, and sectioned sagittally at 6 μm. Sections were stained with Safranin O/Fast Green and subjected to immunohistochemical analysis to assess cartilage damage. As shown in **Fig. 3a**, 12m-old $Grn^{-/-}Gba1^{9V/9V}$ mice exhibited markedly more severe cartilage degeneration compared with WT, $Gba1^{9V/9V}$, and $Grn^{-/-}$ mice. Immunohistochemical staining further revealed significantly elevated levels of Aggrecan neoepitope and Collagen X in the cartilage of $Grn^{-/-}Gba1^{9V/9V}$ mice relative to the other genotypes (**Fig. 3b**).

Conclusions: In this study, we found that PGRN deletion further impaired lysosomal function in chondrocytes with Gba1 inhibition. In addition, PGRN deficiency led to bone loss and chondrocytes degradation in 12m-old Gba1 mutant mice compared with other controlled mice.

Significance/Clinical relevance: The link between the lysosomal protein PGRN and the lysosomal enzyme GCase provides new insights into lysosomal regulation of chondrocyte metabolism and musculoskeletal disorders. These findings highlight lysosomal regulation as a potential therapeutic target for osteoarthritis and other cartilage degenerative diseases.

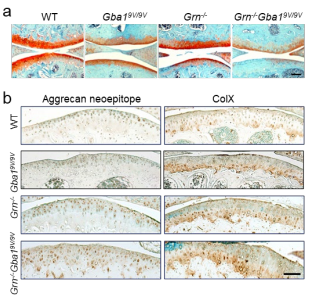

Figure 3. PGRN and Gba1 double mutation further aggravated cartilage degeneration in 12m-old mice. (a) Safranin O staining in knee joint section collected from 12m-old WT, $Gba1^{9V/9V}$, $Grn^{-/-}$ and $Grn^{-/-}Gba1^{9V/9V}$ mice. (b) Immunohistochemical staining of Aggrecan neoepitope and Collagen X of knee joint section collected from 12-month-old WT, $Gba1^{9V/9V}$, $Grn^{-/-}$ and $Grn^{-/-}Gba1^{9V/9V}$ mice.

Reference:

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2. Zhao, X., et al., PGRN deficiency exacerbates, whereas a brain penetrant PGRN derivative protects, GBA1 mutation-associated pathologies and diseases. Proc Natl Acad Sci U S A, 2023. 120(1): p. e2210442120.