Video Article

Establishment of a Surgically-induced Model in Mice to Investigate the Protective Role of Progranulin in Osteoarthritis

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Abstract

Destabilization of medial meniscus (DMM) model is an important tool for studying the pathophysiological roles of numerous arthritis associated molecules in the pathogenesis of osteoarthritis (OA) *in vivo*. However, the detailed, especially the visualized protocol for establishing this complicated model in mice, is not available. Herein we took advantage of wildtype and progranulin (PGRN)-/- mice as examples to introduce a protocol for inducing DMM model in mice, and compared the onset of OA following establishment of this surgically induced model. The operations performed on mice were either sham operation, which just opened joint capsule, or DMM operation, which cut the menisco-tibial ligament and caused destabilization of medial meniscus. Osteoarthritis severity was evaluated using histological assay (*e.g.* Safranin O staining), expressions of OA-associated genes, degradation of cartilage extracellular matrix molecules, and osteophyte formation. DMM operation successfully induced OA initiation and progression in both wildtype and PGRN-/- mice, and loss of PGNR growth factor led to a more severe OA phenotype in this surgically induced model.

Video Link

The video component of this article can be found at https://www.jove.com/video/50924/

Introduction

Osteoarthritis (OA), also known as degenerative arthritis, affects 15% of the world's population and over 46 million people within the United States, and is characterized by synovitis, cartilage degeneration, and osteophyte formation[1]. It can be a result of a complex interplay of genetic, metabolic, biomechanical and biochemical factors. The underlying mechanisms of OA continue to evade the scientific community. There are presently numerous animal models which can mimic the pathogenesis of OA[2,3]. It is important to establish animal models in mice because of both the availability of various genetically modified mice and the cost effectiveness of experimentation. Among the different kinds of experimental OA models, the surgically induced destabilization of medial meniscus (DMM) model is a well-accepted OA model because of its good reproducibility and a relatively slower progression during OA development. Both of these attributes have been key for the evaluation of OA progression in different treatments or transgenes[3-8]. However, the consistency of surgical OA model is affected by various factors during the surgery and as a result, the application of surgical mouse model is limited.

Progranulin (PGRN) is a multi-functional growth factor expressed in various cells. It is known that PGRN plays a critical role in various physiological and disease processes such as wound healing [9], tumorigenesis[10], and inflammation[11-15]. Studies also shown that insufficiency of PGRN can cause degenerative diseases of nervous system in both humans and mice[16-18]. It is known that PGRN is expressed in human articular cartilage, and its level is significantly elevated in cartilage of patients with OA and rheumatoid arthritis[19]. In addition, PGRN also plays a crucial role in chondrocyte proliferation [20], differentiation and endochondral ossification of growth plate during development [21,22]. Recently, we reported that PGRN antagonized TNF-α through binding to TNF receptors and exhibited an anti-inflammatory function in inflammatory arthritis models[13,14,23,24]. However, the role of PGRN in OA, especially *in vivo*, remains to be an enigma. Herein, we present the procedure to induce a surgical DMM model, and investigate the role of PGRN in OA development through establishing DMM model in WT and PGRN-/- mice.

Protocol

All of the surgical procedures relating to the animals should be approved by local Institution's Animal Care and Ethics Committee, with an effort made to minimize pain and discomfort caused by the surgery.

1. Preparation

1. Select 8-12 weeks old male C57BL/6 mice with a body weight of approximately 25 g for surgery.
2. Anesthetize the animals through intraperitoneal injection of a cocktail containing both xylazine (5 mg/kg) and ketamine (40 mg/kg).
3. Shave the knee with razors, then surgically drape the animal and sterilize the surgical site with betadine and alcohol (3x) and cover the mouse eyes with ointment.

2. Surgical Process

1. Make a 1 cm longitudinal medial para-patellar incision to expose knee joint.
2. Open the knee joint gently through lateral dislocation of the patella and patellar ligament.
    1. Cut the knee joint capsule longitudinally through the medial para-patellar incision in step 2.1.
    2. Dissect knee joint capsule with forceps.
    3. Grab the distal part of hind paw with left hand, and perform lateral dislocation of the patella and patellar ligament with forceps. Hold the hind paw gently and make sure to avoid trauma in the paw. The better the joint capsule is dissected, the less force will be required to make the dislocation.
3. Drip sterile saline on the surface of articular cartilage during operation to avoid drying out of cartilage surface.
4. Cut through the medial meniscotibial ligament which anchors medial meniscus to the tibial plateau. Avoid injuring the cartilage beneath the medial meniscus.
    1. Identify the medial meniscus which locates between medial condyle of femur and medial plateau of tibia.
    2. Identify the meniscotibial ligament which connects lateral side of medial meniscus with intercondylar eminence of tibia.
    3. Hold the hind paw gently with hand, and cut through the medial meniscotibial ligament carefully by using a No. 10 surgical blade. Make sure not to cause injure to articular cartilage and other ligaments. In many cases, the covering fat pad needs to be pulled to the side but not to be removed in order to identify the ligament.
5. Close the knee joint capsule with a 6-0 absorbable suture.
6. Close the skin with 6-0 silk suture.

3. Post-operative Care

1. Apply one drop of 0.25% bupivacaine in the surgical site of each mouse to minimize post-operative pain.
2. Leave the operated mice free to get water and food.

4. Histological Scoring of Surgical DMM Model

1. Sacrifice the mice at indicated time points (*e.g.* 4 weeks, 8 weeks, and 12 weeks. Here we showed results of 8 weeks as representative) after the surgery.
2. The entire knee joints are fixed, decalcified, embedded by paraffin and then sectioned serially at 5 μm interval.
    1. Cut the whole hind limbs with No. 10 blades.
    2. Dissect the skin and muscles of the hind limbs carefully, and fix the samples with 4% PFA for 3 days in RT.
    3. Remove the PFA, and clean the samples with water. Afterwards, decalcify the samples in EDTA at 4 °C for 2 weeks.
    4. Dehydrate the samples in an ethanol gradient. In detail, keep the samples in 70% ethanol for 1 hr, then change the ethanol and keep the samples in a new set of 70% ethanol for O/N. Afterwards, put the samples in 80% and 90% ethanol consequently, and keep them for 1 hr, respectively, followed by 100% ethanol for O/N.
    5. Remove the ethanol. Keep the samples in oxylene for 1 hr, and change to a new set of oxylene. Repeat this step 3x.
    6. Embed the samples into a paraffin mold using the Leica embedding center. Afterwards, the samples are sectioned at 5 μm using a rotary microtome (Leica RM2255, Germany) and then collected onto glass slides.
    7. Serial sagittal sections are cut for each sample spanning a region from the center of the lateral condyle to the center of the medial condyle.
3. Safranin O staining is performed, followed by scoring and statistical analysis through OARSI scoring system as described previously[25].
    1. First, deparaffinize the slides in oxylene, and hydrate them in an ethanol gradient to distilled water. Thereafter, the slides are stained through Weigert's Iron Hematoxylin Solution, 0.05% Fast Green (FCF) Solution, 1% Acetic Acid Solution, and 0.1% Safranin O Solution. Mount the slides using resinous medium.
    2. Score the Safranin O stained slides based on loss of proteoglycan (red color) in cartilage and percentage of destruction in cartilage structure, do statistical analysis for the histological score.

Representative Results

DMM model was successfully established in mice, and deficiency of PGRN exaggerated surgically-induced OA development.

Sham and DMM operations (**Figure 1**) were performed in WT and PGRN-/- mice. 8 weeks after operation, the mice were sacrificed, and Safranin O staining was performed on the sections from knee joints, followed by statistical analysis of arthritis score based on histology. As shown in **Figure 2A**, there was no obvious degeneration of cartilage in both genotypes in sham operation groups based on Safranin O staining, which implied the similar baseline conditions (upper panels). 8 weeks after DMM operation (lower panels), there was loss of proteoglycan and

destruction of cartilage structure in both WT and PGRN-/- mice (red arrows). Both the loss of proteoglycan and degradation of cartilage suggest the successful induction of the OA phenotype. The bars of the columns indicated the variation between intra group members following DMM operation. However, the bar is relatively short, and PGRN knockouts displayed a higher loss of proteoglycan and the cartilage structure was more severely destroyed in comparison to the wild type breed. Moreover, the statistical analysis for cartilage score was performed as described previously[25], and cartilage score was significantly higher in PGRN knockouts than the WT group. Our results demonstrated that a loss of PGRN resulted in more severe OA phenotype.

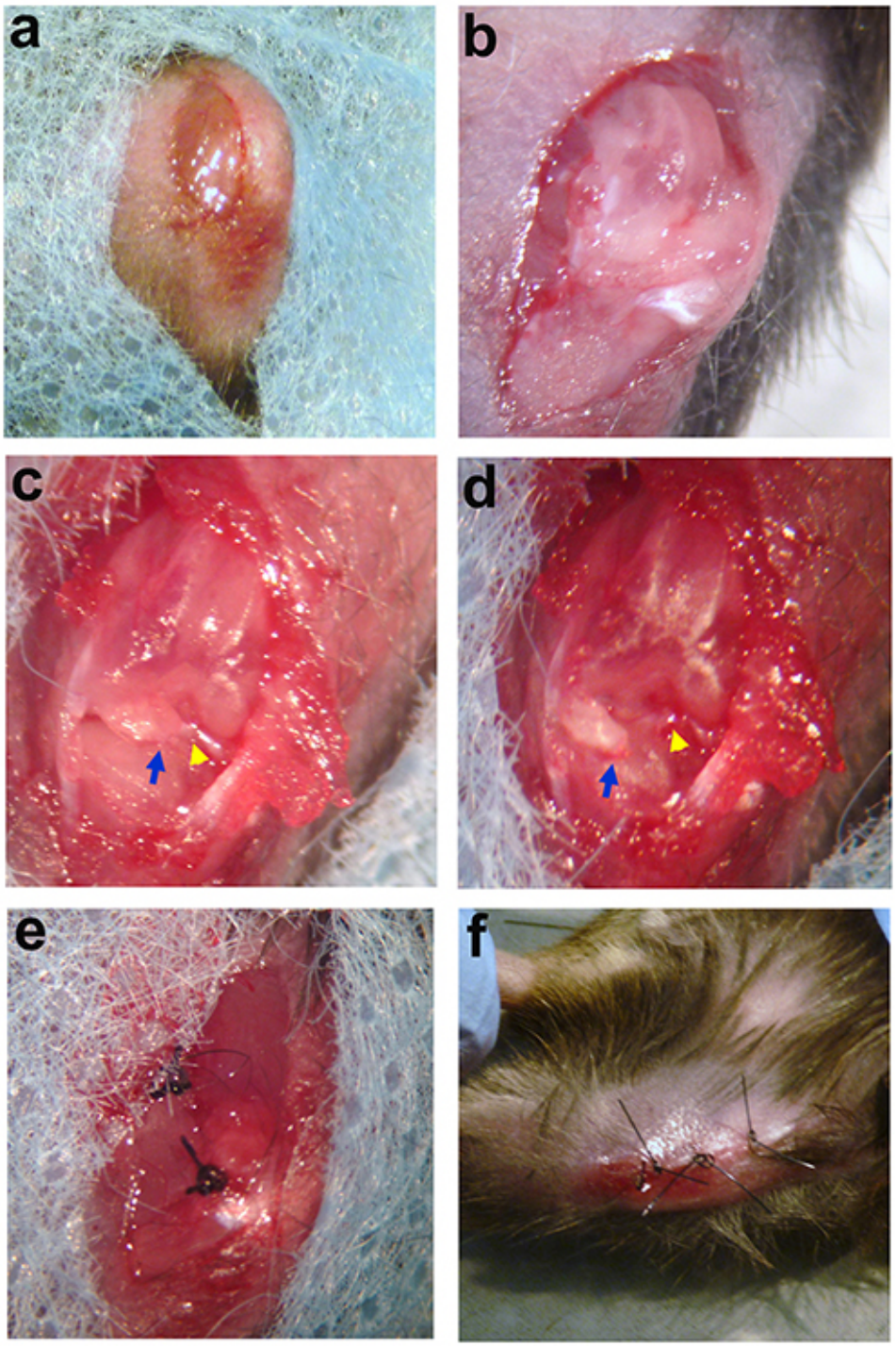

Figure 1. Surgical procedure for DMM induction. a. A medial para-patellar incision is made in skin of knee joint. **b.** Exposure of knee joint space after lateral dislocation of joint capsule. **c.** Inner structure of knee joint. Blue arrow indicates medial meniscus. Yellow arrow shows meniscotibial ligament. **d.** Medial meniscus is destabilized. Blue arrow indicates the medial meniscus which is dislocated from original site. Yellow arrow shows residual part of meniscotibial ligament which has been dissected. **d.** The joint capsule is closed. **f.** The skin is closed. Click here to view larger image.

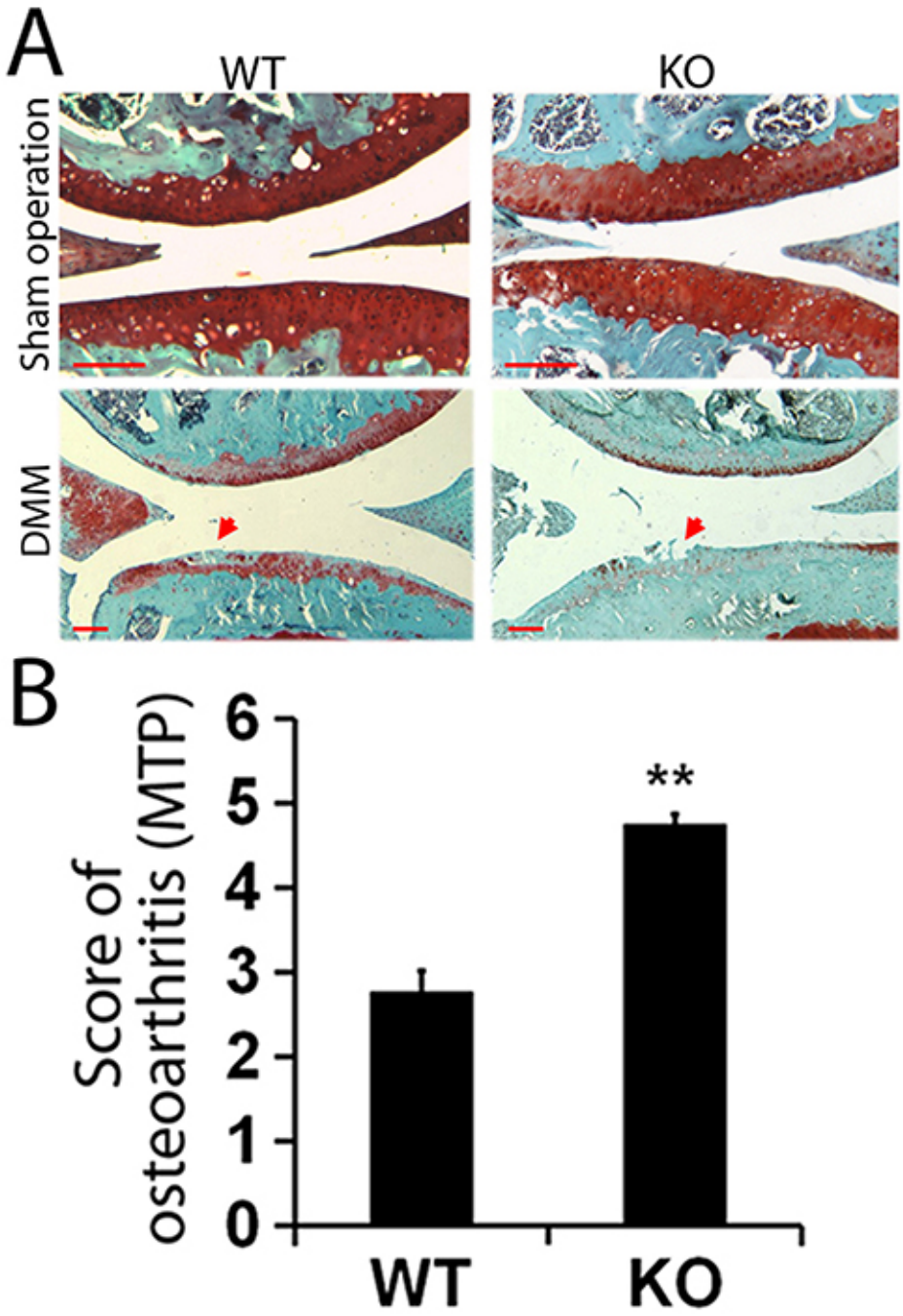

Figure 2. Representative pictures of histology and statistical analysis for score of osteoarthritis. A. Representative histological pictures of WT and PGRN-/- mice 8 weeks following sham operation or DMM, assayed by Safranin O staining. Red arrows indicate cartilage destruction. Loss of red color in cartilage implies loss of proteoglycan. Scale bar, 100 μm. **B.** Statistical analysis for score of osteoarthritis in both PGRN-/- and WT mice. Click here to view larger image.

Discussion

It is reported that the strain of mice is very important for DMM model induction, as different strains of mice have varying severity of OA after DMM, with highest level in the 129/SvEv strain, followed by C57BL6, 129/SvInJ and then FVB/n[26]. A large part of transgenes are established in C57BL6 mice, such as PGRN-/- mice we used in the present study, which are relatively susceptible to DMM. However, if the transgene is based on insensitive strain such as FVB/n mice, it is necessary to backcross these mice with susceptible strains before DMM induction. The gender of mice used for DMM induction is also very important as studies have already shown that female mice are less sensitive than male mice[27]. Moreover, the age of mice for DMM operation varies from 8 weeks old to 12 weeks old based on literature[29], which also plays a critical role in pathogenesis of OA. Therefore, mice of the same age should be operated to eliminate this effect. Post operative pain control is required to enhance the movement of mice after surgery. As DMM model leads to OA through movement mediated wear and tear in cartilage, inadequate movement as a result of post operative pain may cause inconsistency of OA development. Currently there is a discrepancy concerning the

definition of DMM regarding whether or not to remove the medial meniscus[4,28]. In our present study, we merely destabilized medial meniscus but did not remove it. Lesions in the DMM model were mainly located on the central weight-bearing region of medial tibial plateau, and the severity of lesions increased during the time course (unpublished data), and the results were consistent with the previous reports[5]. Furthermore, the OARSI score following DMM operation was relatively consistent between different members of the same genetic group.

PGRN plays an important role in cartilage development and arthritis[14,21].We previously reported that PGRN interplays with metalloproteases ADAMTS-7 and ADAMTS-12, and protects cartilage matrix protein COMP from degradation[19]. Moreover, it is well-established that TNF-α plays a critical role in cartilage destruction[24]. Recently, we found that PGRN antagonized TNF-α and protected against cartilage destruction in inflammatory arthritis models[14]. In this study, we induced DMM model in WT and PGRN-/- mice, and as expected, PGRN-/- mice exhibited exaggerated progression of OA following induction of DMM, which was indicated by more severe loss of proteoglycan in Safranin O staining and significantly higher arthritis score based on histology (**Figure 2**). Moreover, the expressions of catabolic markers were dramatically elevated in PGRN-/- group (unpublished data), suggesting accelerated OA progression in deficiency of PGRN.

In conclusion, our protocol for establishment of DMM model successfully induced OA phenotype in WT and PGRN-/- mice, and the induction was consistent and reproductive. The successful generation of DMM model in C57/B6 mice provides a useful tool for evaluating therapeutic agents and OA related genes for OA in future. In the current study, the result of DMM model documents a protective role of PGRN in OA development.

Disclosures

We herein declare that we have no conflict of interest.

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