MMP-9 Modulates Joint Inflammation and Cartilage Degradation after Overload Injury in the Mouse Using a Novel Model

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Introduction: Joint kinematics plays a key role in the development of primary and secondary osteoarthritis (OA). A disruption of normal kinematics, such as ACL injury, due to sports injury or surgical intervention can result in spontaneous OA and cartilage degradation within months even weeks after initial damage. Several recent studies in mice have introduced joint instability to study OA development at molecular levels using mouse with a deletion of specific gene. It is apparently important as well to know how the gene knockout affects cartilage degradation and repair without an alteration of joint kinematics, which orthopedic surgeon strives to restore in clinics. Of particular interests is MMP-9, which is important for long-bone formation and the recruitment of pro-inflammatory cells in synovial membrane that often initiates a cascade of degradative events through several pro-inflammatory cytokines including interleukin 1 (IL-1) and tissue necrosis factor alpha (TNF-α). Using a transgenic knockout technique, Vu et al found an increase in hypertrophic zone of growth plate cartilage and a delayed formation of secondary (epiphyseal) ossification center in MMP-9-deficient mice at birth [2]. At 3 weeks after birth, there were no noticeable musculoskeletal changes except shorter long bones in the MMP-9-deficient mouse. The objective of this study was to establish a murine model to study load injury in cartilage without alternating joint kinematics, and to determine whether the deletion of the MMP-9 gene (+/−) in the mouse as compared to wildtype (+/+) affects cartilage degradation at 8 weeks post-injury.

Materials and Methods: Twenty eight MMP-9 knockout (FVB.Cg-Mmp9tm1Tvu/J) and matching wildtype (FVB/NJ) mice (7 male and 7 female in each group) were obtained from Jackson Laboratories (Bar harbor, Maine). The animals were maintained in the animal facility at HSS for one week before surgery using established protocols approved by the IACUC. All the mice were 8 weeks old (musculoskeletal mature) at the time of surgery. The right knee of each animal was operated and injured with the following procedures. A medial parapatellar arthroscopy was used to expose the medial femoral condyle with an 8–10 mm incision in the midline center of the knee. An indenter (0.5 mm x 2mm) was positioned perpendicular to the articular surface and injured at the femoral trochlear region with static stress of 6 MPa for 4 minutes (20~40% cell death) based on our previous in vitro study [3]. The patella was then re-aligned and the wound was closed in layers. The same surgical procedure with no load-injury was performed in the contralateral knee to serve as a sham control. The animals were sacrificed at 8 weeks after operation. Collagen cleavage and proteoglycan loss was determined using immunofluorescence (C1,2C Mab, Ibex) and safranin-O/fast green while collagen-network integrity (birefringence) was determined by polar-light microscope. Joint inflammation was determined by the thickening of synovial membrane (H&E), recruitment of macrophages(F4/80 Mab), and upregulation of TNF-α.

Results: All the animals were able to move freely one day after surgery and gained full locomotion (climbing cage and other activities) at 3–6 days post operation. No significant difference in animal behavior was noted between wildtype and MMP-9 knockout mice. All the joints appeared normal without apparent infection or swelling at the time of sacrifice. Hematoxylin & cosin staining showed a loss of PG in the injured joints as compared to the sham control for both wildtype and MMP-9 knockout mice. This suggests cell death was localized in the injured joints. The disappearance of chondrocytes was consistent with a loss of proteoglycan as determined by safranin-O/fast green staining. Along with the increase of PG loss/degradation was significant thickening of the synovial membrane as compared to sham-operated joints in the wildtype mice (p<0.001). Further thickening of the synovial membrane was also found in the MMP-9 KO mice as compared to that of the wildtype (p<0.01). An increased MMP-9 staining was found in the injured wildtype joint as compared to its contralateral knee. An increase of PG loss and collagen cleavage along with a loss of collagen integrity (birefringence) was found in the injured joint. In the injured joint, a decreased collagen cleavage along with increased birefringence was found in the MMP-9 KO mouse suggesting that MMP-9 may play a role in collagen degradation. Some RA-like inflammation (overgrown synovial coverage and synovium invasion into cartilage) was found in load-injured joints of MMP-9 knockout mice. This finding is correlated with recruitment of macrophages and increased accumulation of TNF-α in the MMP-9 KO mice.

Discussion: In this study, we successfully created a murine model to study cartilage degradation after injury without alternating joint kinematics (versus ACL transection and meniscectomy). We found that cell death/loss was co-localized with matrix degradation and PG loss in articular cartilage 8 weeks after injury. A thickening of synovial membrane along with increased staining of MMP-9 in the injured joint indicates joint inflammation. Considering the departure of acute inflammatory responses at 8 weeks after surgery, our finding suggests chronic joint inflammation plays a role in injury-induced cartilage degradation. In the MMP-9 knockout mouse, a further increase of cartilage degradation along with RA-like inflammation (pannus and synovium invasion into cartilage) was found. This finding was quite unexpected and due possibly to an upregulation of other molecules, such as MMP-2 and MMP-13. Further studies to identify specific molecular and structural changes in cartilage and synovial membrane are needed. In summary, our results suggest that modulating joint inflammation after load-injury is important for cartilage repair/degradation and MMP-9 plays an important role in regulating inflammation and chronic repair of cartilage after load injury.


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Figure 1. Safranin-O/fast green staining of proteoglycan in sham-operated and load-injured cartilage of wildtype and MMP-9 knockout mouse 8wks post injury. Bar=100 µm

Figure 2. Increased staining (green) of collagen cleavage in load-injured wildtype cartilage by C1,2C MAb as cell nucleus stained blue.