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Response of knee fibrocartilage to joint destabilization

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SUMMARY

Objective: A major challenge to understanding osteoarthritis (OA) pathology is identifying the cellular events that precede the onset of cartilage damage. The objective of this study is to determine the effect of joint destabilization on early changes to fibrocartilage in the joint.
Design/Methods: The anterior cruciate ligament was transected in collagen reporter mice (Col1CFP and ColXRFP). Mineralization labels were given every 2 weeks to measure new mineralized cartilage apposition. Novel fluorescent histology of mineralized tissue was used to characterize the changes in fibrocartilage at 2 and 4 weeks post-injury.
Results: Changes in fibrocartilaginous structures of the joint occur as early as 2 weeks after injury and are well developed by 4 weeks. The alterations are seen in multiple entheses and in the medial surface of the femoral and tibial condyles. In the responding entheses, mineral apposition towards the ligament midsubstance results in thickening of the mineralize fibrocartilage. These changes are associated with increases in ColX-RFP, Col1-CFP reporter activity and alkaline phosphatase enzyme activity. Mineral apposition also occurs in the fibrocartilage of the non-articular regions of the medial condyles by 2 weeks and develops into osteophytes by 4 weeks post-injury. An unexpected observation is punctate expression of tartrate resistant acid phosphatase activity in unmineralized fibrochondrocytes adjacent to active appositional mineralization.
Discussion: These observations suggest that fibrocartilage activates prior to degradation of the articular cartilage. Thus clinical and histological imaging of fibrocartilage may be an earlier indicator of disease initiation and may indicate a more appropriate time to start preventative treatment.

Introduction

Degenerative joint disease leading to incapacitating osteoarthritis (OA) is a chronic process that is the endpoint of genetic and environmental conditions[1], and is a major financial burden of health care[2–4]. Common to many forms of OA is a past history of trauma leading to joint instability that eventually degrades the articular cartilage. Animal models that are based on induced joint instability require a number of weeks to express the degenerative changes, and these changes are often associated with osteophytes, which further alter the function of the joint[5,6]. While there are numerous studies examining the influence of inflammatory cytokines and metalloproteases on the destruction of articular cartilage, less work has focused on other changes to the joint organ that precede and may predict the progression of articular cartilage damage. Some of the impediments for detecting early changes are inherent to paraffin embedded histology, which requires the use of decalcified tissue[7] and cannot accommodate fluorescent signals.

We have developed several GFP reporters that are expressed in chondrocytes and improve the sensitivity of histological evaluation of articular cartilage and fibrocartilaginous structures. In this study, a double reporter mouse was employed. A type X collagen reporter (ColX-RFPchry) is expressed in cells within the mineralizing regions of articular, fibrocartilage and endochondral cartilage. A type I collagen reporter (Col3.6-CFP) is expressed in fibroblasts, osteoblasts and fibrochondrocytes, but not articular or endochondral chondrocytes. We have also developed a cryohistological approach that maintains GFP signals and enzymatic

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activity in mineralized tissues. The method is based on cryotape that adheres to the tissue section. Therefore, the coverslip can be easily removed and multiple rounds of staining and imaging can be performed, leading to colocalization of several response measures on a single section. We employ this approach in the current study to investigate the pathogenesis of OA in the knee joint organ[8], with specific focus on fibrocartilage mineralization during osteophyte formation that is often associated with knee destabilization models[9].

This study employs a histological strategy for consistently sectioning and comparing normal vs destabilized knees taken from the same GFP reporter mouse. The objectives are to determine the spatiotemporal changes to collagen (Col3.6-CFP and ColX-RFPchry) reporters, mineral apposition, enzymatic indicator of mineralization (alkaline phosphatase, AP), tissue remodeling (tartrate resistance acid phosphatase, TRAP), and cartilage proteoglycan distribution [toluidine blue (TB) staining] during early changes (up to 4 weeks) following joint destabilization in the mouse. We believe understanding the molecular basis for these dramatic changes and how they could directly or indirectly influence the neighboring articular cartilage may contribute to understanding the pathogenesis of OA.

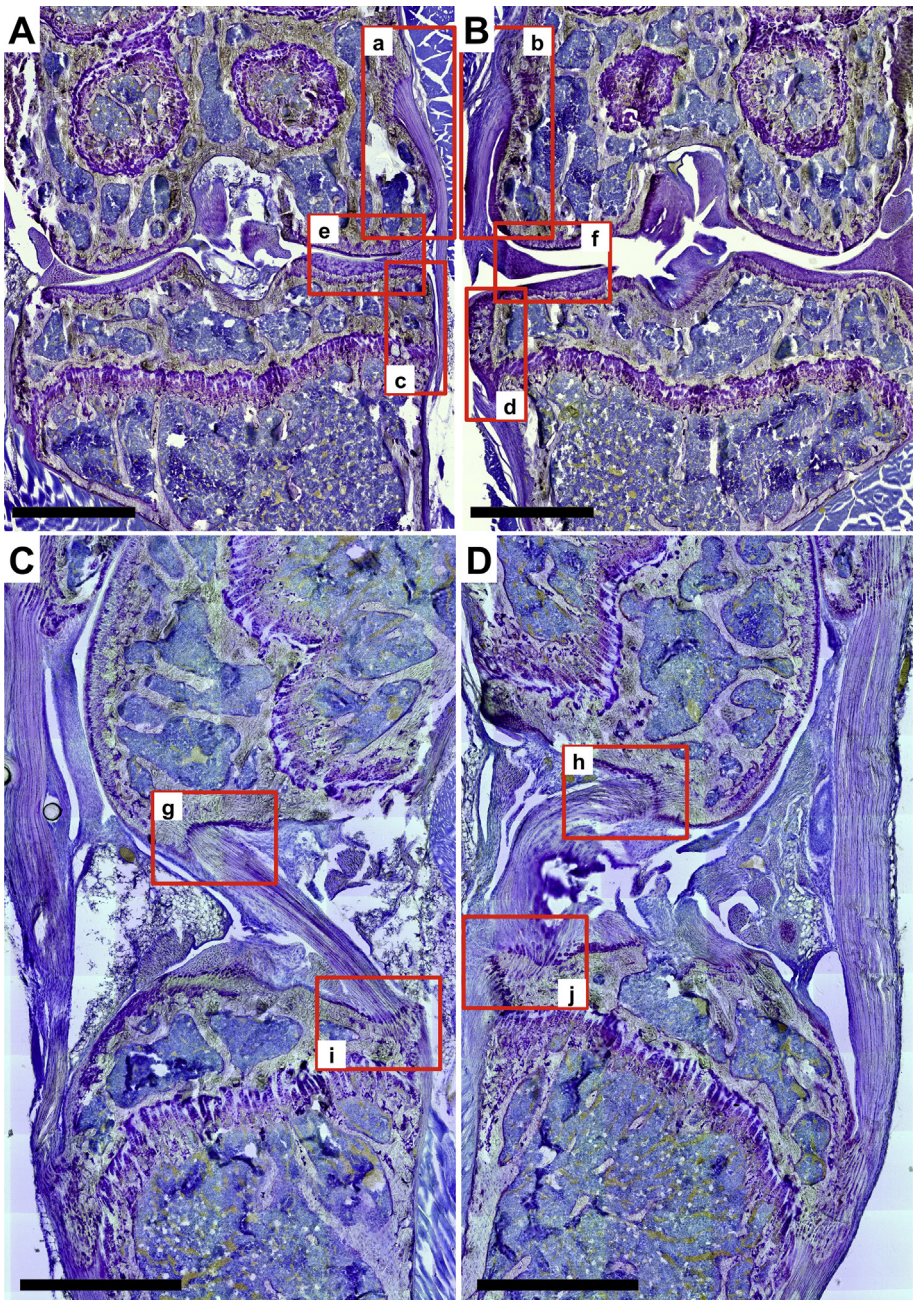

Fig. 1. A,B: Coronal sections of the sham (A) and ACL-transected (B) knees are taken from the same animal at 4 weeks post-injury. Three ROI will be presented in Figs. 2–4: a/b is the femoral enthesis of the MCL with the medial femoral condyle (Fig. 2). c/d is the medial tibial condyle located beneath the MCL (Fig. 3). e/f is the articular cartilage and overlying meniscus (Fig. 4). **C,D:** Sagittal sections of the sham (C) and ACL-transected (D) knee from the same animal at 4 weeks post-injury. Two ROI will be presented in Fig. 5. g/h is the femoral attachment site of the PCL [Fig. 5(A)–(B)]. i/j is the tibial attachment site of the PCL [Fig. 5(C)–(D)]. Scale bar – 1 mm.

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