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Editorial

The importance of performing knee surgery in rats

Approximately 200,000 anterior cruciate ligament (ACL) injuries occur each year in the United States, with approximately half of these leading to ACL reconstruction surgery[1]. ACL injuries trigger a biological response that includes cell death/apoptosis and a surge of inflammatory cytokines and degradative enzymes such as cathepsin proteases and matrix metalloproteinases (MMPs)[2]. ACL deficiency also typically results in biomechanical changes in the joint, including anterior–posterior (AP) and rotational instability in the knee, which is strongly correlated with subsequent cartilage degeneration and development of post-traumatic osteoarthritis (PTOA)[3]. The relative contributions of these biological and biomechanical changes to PTOA initiation and progression remain unclear.

To investigate the biological and biomechanical factors that contribute to PTOA, rodent models are often used to study the pathogenesis and treatment of the disease on a compressed timescale. These models are quite useful for studying genetic factors, specific cellular processes, and the time course of microstructural changes within the joint. However, rodent models also have some notable limitations. Joint instability is a particularly important factor in rodent models of PTOA that involve disruption of the ACL or other joint structures such as the medial meniscus. Mice and rats are prey animals (and must hide lameness), so they will resume relatively normal cage activity and joint loading immediately following injury[4], instead of undergoing a period of decreased loading and rehabilitation as in human patients. This immediate joint mobilization likely exacerbates injury-induced inflammation, aggravates injured joint tissues, and generally produces an injury response more severe than that of humans. Given these notable differences in mobilization, it is a worthwhile endeavor to investigate post-injury conditions that more closely replicate human joint biomechanics following ACL injury and may more accurately reproduce the mechanisms that lead to PTOA progression. However, to date few rodent studies of PTOA have taken these biomechanical factors into account.

ACL reconstruction surgery in humans is typically very successful at restoring knee stability, knee kinematics, and functional scores[5,6]. However, ACL injury is still associated with a considerably higher risk of developing PTOA in the injured joint whether ACL reconstruction was performed or not[7,8]. In rodent models of PTOA, it is extremely challenging to fully replicate human ACL reconstruction surgery, which presents a challenge for isolating the effects of altered biomechanics on joint degeneration. However, Murata *et al.* have addressed this challenge by developing a simple extracapsular surgical method to restabilize injured knee joints in rats following ACL transection[9–12]. This surgical method does not fully replicate human ACL reconstruction, but is able to limit AP joint laxity and maintain the normal articulation of the injured joint. Their previous studies using this method found that joint restabilization performed at the same time as ACL transection in rats was able to slow articular cartilage degeneration[9–11], suppress tumor necrosis factor-alpha (TNF-α) and caspase-3 expression in the joint[9], inhibit osteophyte formation[10], reduce expression of MMP-13 in articular cartilage[11], and promote joint healing[12] post-surgery relative to ACLT joints that were not restabilized. Based on these promising results, this surgical restabilization method could be a tremendously useful tool for investigating biomechanical vs biological processes affecting PTOA progression after ACL injury.

The current study by Murata *et al.* utilizes their novel joint restabilization method to investigate mechanisms of osteophyte formation following ACL transection. Osteophytes are a hallmark of osteoarthritic joints that are thought to form in response to joint instability. Osteophytes begin as cartilaginous growths (chondrophytes), which can form rapidly following a joint injury and act to stabilize an injured joint[13]. Subsequently, these chondrophytes undergo intramembranous and endochondral ossification to form mature osteophytes, which are mineralized and fully integrated with native bone and articular cartilage[14]. Osteophyte formation is strongly affected by biomechanical factors; mechanical unloading following joint injury considerably diminishes the formation of osteophytes[15]. Osteophyte formation is also modulated by biological factors, in particular transforming growth factor beta (TGF-β) and bone morphogenetic protein 2 (BMP-2)[16] from the synovium. Accordingly, the goal of this study was to investigate osteophyte formation induced by TGF-β-Smad2/3 and BMP-2-Smad1/5 signaling in ACL-deficient rat knees with or without knee restabilization. Consistent with previous studies by this group, ACL transection in rats induced considerable AP joint laxity, articular cartilage degeneration, and osteophyte formation in the injured joint. Additionally, synovial cell hyperplasia and proliferation, synovial thickening, and increased TGF-β and Smad2/3 expression were observed in ACLT joints. All of these outcomes were significantly reduced in restabilized joints. These findings substantiate the role of joint instability in osteophyte formation, and establish this joint restabilization method as a novel and effective tool for modulating joint biomechanics in rodent models of PTOA.

The novel surgical knee restabilization method pioneered by this group represents a larger opportunity for advancing studies of PTOA using mice and rats. A majority of the methods currently used to initiate PTOA in rodents involve destabilization of the joint by disrupting the ACL, the medial meniscus, or other joint structures. This makes it difficult to determine the extent to which joint degeneration is driven by biological vs biomechanical factors. In fact, for some injury models, biomechanical instability can "overpower" potential therapies, resulting in development of PTOA regardless of treatment. As a consequence, the efficacy of targeting

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specific aspects of the injury response may be clouded by instability and mechanical damage to the joint. For example, in our previous study investigating the effects of alendronate (ALN) treatment on PTOA progression following non-invasive ACL injury[17], we found that ALN was chondroprotective in injured mouse joints at 4 weeks post-injury, but joint degeneration progressed to severe PTOA in all mice by 8 weeks post-injury regardless of treatment.

By utilizing the surgical restabilization method described in this article (or comparable methods), researchers would be able to individually assess the injury response and potential treatments with minimal contributions due to joint instability. Importantly, it is likely that this method could be used in mice to isolate the effect of joint biomechanics on PTOA progression in various genetic models. In fact, Arce *et al.* used a similar extracapsular restabilization method in mice with ACL transection, and found that restabilization diminished articular cartilage damage at 10 weeks post-surgery[18]. Overall, this surgical restabilization method is a promising research tool that could have a tremendous impact on understanding the pathogenesis and treatment of PTOA, and advances rodent models of PTOA to be more relevant to the treatment human patients.

Conflict of interest

None.

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