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Comparison of mouse and human ankles and establishment of mouse ankle osteoarthritis models by surgically-induced instability

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SUMMARY

Objective: Prevalence of ankle osteoarthritis (OA) is lower than that of knee OA, however, the molecular mechanisms underlying the difference remain unrevealed. In the present study, we developed mouse ankle OA models for use as tools to investigate pathophysiology of ankle OA and molecular characteristics of ankle cartilage.
Design: We anatomically and histologically examined ankle and knee joints of C57BL/6 mice, and compared them with human samples. We examined joints of 8-week-old and 25-month-old mice. For experimental models, we developed three different ankle OA models: a medial model, a lateral model, and a bilateral model, by resection of respective structures. OA severity was evaluated 8 weeks after the surgery by safranin O staining, and cartilage degradation in the medial model was sequentially examined.
Results: Anatomical and histological features of human and mouse ankle joints were comparable. Additionally, the mouse ankle joint was more resistant to cartilage degeneration with aging than the mouse knee joint. In the medial model, the tibiotalar joint was markedly affected while the subtalar joint was less degenerated. In the lateral model, the subtalar joint was mainly affected while the tibiotalar joint was less altered. In the bilateral model, both joints were markedly degenerated. In the time course of the medial model, TdT-mediated dUTP nick end labeling (TUNEL) staining and Adamts5 expression were enhanced at early and middle stages, while Mmp13 expression was gradually increased during the OA development.
Conclusion: Since human and mouse ankles are comparable, the present models will contribute to ankle OA pathophysiology and general cartilage research in future.

Introduction

Osteoarthritis (OA) is the most common joint disorder characterized by cartilage degradation and osteophyte formation[1]. Since knee OA is most prevalent, many experimental studies using human samples or animals have been based on this condition. Particularly, establishment of surgically induced mouse knee OA models in 2005 made a major breakthrough in molecular pathophysiological research of OA[2,3]. Many studies based on mouse knee OA models and genetically modified mice have revealed that various kinds of molecules and signals including proteolytic enzymes, endochondral ossification-related molecules, and inflammatory factors, affect OA development[4].

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Among lower limb joints, articular cartilage of the ankle is more resistant to degeneration than that of the knee[5]. Although the prevalence of knee OA is about 15–20% in an adult population, that of ankle OA is about 1%[6–8]. Previous studies showed that several factors including the joint structure, biomechanical property, and chondrocyte phenotype determine differences between the two joints. First, each layer of articular cartilage in the ankle is thinner than that in the knee[9]. In biomechanical research, the ankle cartilage shows higher dynamic stiffness than the knee cartilage[10]. In explant culture, the ankle cartilage is less responsive to catabolic stimulation than the knee cartilage[11]. However, the characteristics of chondrocytes in the two joints have not been thoroughly compared from the aspect of molecular biology.

Despite its lower prevalence, ankle OA is a painful joint disease that affects a patient's quality of life as much as hip arthrosis, end-stage kidney disease, or congestive heart failure[12–16]. The morbidity rate of ankle OA has been increasing due to the increasing number of sports injuries and elderly people[17–19]. Although total joint replacement has become the most standard and excellent treatment for severe OA patients in parallel with the improvement of implants, results of total ankle replacement are still inferior to those of knee or hip joint replacement[13,16,20–25]. Arthrodesis is still a popular method for severe ankle OA, but limited range of motion is inevitable. To resolve these problems, pathophysiology of ankle OA should be elucidated first. Most cases of ankle OA are regarded as post-traumatic[7]; however, an *in vivo* experimental model to reproduce post-traumatic ankle OA has not yet been established.

To learn whether a mouse ankle joint is appropriate as an experimental model for a human ankle, we first examined the anatomical structure of an ankle joint of C57BL/6, a representative inbred strain of laboratory mice, and compared cartilage degeneration of mouse ankle and knee joints with aging. After confirming the similarity of the ankle joints of both species, we further established mouse ankle OA models by surgical induction of instability as research tools to investigate characteristics of ankle chondrocytes, and to study molecular mechanisms of articular cartilage homeostasis by comparison of the ankle and the knee.

Methods

Human samples

We obtained human cartilage samples from OA individuals undergoing total knee or ankle arthroplasty after obtaining written informed consent as approved by the Ethics Committee of the University of Tokyo. Articular tissues in less degenerated lesions were used for histological analyses.

Mouse ankle OA model

We performed all experiments according to a protocol approved by the Animal Care and Use Committee of the University of Tokyo. C57BL/6 mice were used for all mouse experiments (mean body weight; 20.5 g, range; 18.2–24.7 g). All mice were placed in plastic cages with sawdust bedding in a specific pathogen free facility, with four to five animals per cage. The room had a 12-h dark/light cycle and was at a constant temperature (18–22°C). Mice were allowed to move freely in the cages and had free access to food and water.

For the aging model, 25-month-old mice were sacrificed (three males, two females). For experimental models, 8-week-old mice were used (six males, six females for each model). Under general anesthesia using 2–3% isoflurane, resection of ligaments and tendons was performed using a surgical microscope. For the medial model, the skin overlying the medial aspect of the left ankle was incised longitudinally to expose the ankle joint. Tibialis posterior tendon [TP, black arrows in the left panel of Fig. 1(A)], which runs along the posterior aspect of the medial malleolus, was pulled out with pinpoint forceps and excised from the insertion of the navicular bone to 5 mm proximal to the ankle joint. Deltoid ligament [DL, green arrows in the left panel of Fig. 1(A)], which originates from the anterior aspects of the medial malleolus to the navicular bone and the talus, was excised at its attachment sites. After removal of TP and DL, the medial ankle capsule, which attaches from the inferior aspects of medial malleolus to medial side of the talus, was incised (Supplementary video 1). For the lateral model, we used the techniques similar to those previously described[26]. The skin overlying the lateral aspect of the left ankle was incised longitudinally to expose the ankle joint. Calcaneofibular ligament [CFL, green arrows in the right panel of Fig. 1(A)], which connects from the apex of the fibular malleolus to the lateral surface of the calcaneus, was excised at its attachment sites. Anterior talofibular ligament [ATFL, blue arrows in the right panel of Fig. 1(A)], which runs from the distal anterior tip of the fibula to the lateral talar neck, was also excised at its attachment sites. The lateral ankle capsule, which connects from anterior aspects of fibula to the lateral side of the talus, was incised after removal of CFL and ATFL (Supplementary video 2). For the bilateral model, in addition to TP, DL, ATFL, CFL and ankle capsules, peroneus longus/brevis tendons [PL/PB, black arrows in the right panel of Fig. 1(A)], which run along the posterior aspects of fibula, were pulled out with pinpoint forceps and excised from the base of fifth metatarsal bone to 5 mm proximal to the ankle joint after skin incision on both sides of the left ankle (Supplementary video 3). The surgical wound was irrigated with saline and closed.

Supplementary videos related to this article can be found online at http://dx.doi.org/10.1016/j.joca.2015.11.008.

A sham operation was performed on the opposite ankle using the same skin incision without ligament/tendon resection. We did not use post-surgical analgesic treatment. The mice were sacrificed 8 weeks after the surgery for analyses.

Histological analyses

The human and mouse samples were fixed in 4% paraformaldehyde for 24 h, decalcified in ethylenediaminetetraacetic acid (EDTA) at 37°C on a shaker for 5 days, and embedded in paraffin. Four-μm frontal sections were prepared and stained with safranin O and fast green according to the standard protocol. For analyses of ankle tissues, we used coronal sections around the center of the talus, containing tibiotalar joint, subtalar joint, and lateral malleolus.

For the comparison of cartilage and subchondral bone, we collected medial tibial plateau cartilage of knee joints and talar articular cartilage of tibiotalar joints from human and mouse. We divided the articular cartilage into three parts: anterior, middle and posterior and prepared sections for each part. The cartilage thickness was measured at 5 points per section. Cancellous bone volume (BV/TV) of the subchondral bone was calculated in two independent squares (400×400 μm) set just below the calcified zone of the mouse medial tibial plateau and the talus of tibiotalar joint using BZ II analyzer software in BZ-8100 (KEYENCE, Osaka, Japan). In the same manner, BV/TV was calculated in two independent squares (1.7×1.7 mm) in human medial tibial plateau and talus of tibiotalar joint.

We quantified the severity of the tibiotalar and subtalar joints for ankle OA, and that of medial and lateral compartments for knee OA, using the Osteoarthritis Research Society International