Mechanisms of Osteoarthritis from Animal Models

Osteoarthritis (OA), one of the most common skeletal disorders characterized by cartilage degradation and osteophyte formation in joints, is induced by accumulated mechanical stress; however, little is known about the underlying molecular mechanism. Several experimental OA models in mice by producing instability in the knee joints have been developed to apply approaches from mouse genetics. Although proteinases like matrix metalloproteases and aggrecanases have now been proven to be the principal initiators of OA progression, clinical trials of proteinase inhibitors have not been successful for the treatment, turning the interest of researchers to the upstream signals of proteinase induction. These signals include undegraded and fragmented matrix proteins like type II collagen or fibronectin that affects chondrocytes through distinct receptors. Another signal is pro-inflammatory factors that are produced by chondrocytes and synovial cells; however, recent studies that used mouse OA models in knockout mice did not support that these factors have a role in the central contribution to OA development. Our mouse genetic approaches found that the induction of a transcriptional activator Runx2 in chondrocytes under mechanical stress contributes to the pathogenesis of OA through chondrocyte hypertrophy. In addition, chondrocyte apoptosis has recently been identified as being involved in OA progression. We hereby propose that these endochondral ossification signals may be important for the OA progression, suggesting that the related molecules can clinically be therapeutic targets of this disease.

**Keywords:** osteoarthritis • chondrocytes • hypertrophy • apoptosis • endochondral ossification • cartilage

Osteoarthritis (OA) and the experimental mouse models

Osteoarthritis (OA), which affects all joints of the body, is characterized by two aspects: one is cartilage degradation shown as a joint space narrowing on radiographs, and the other is osteophyte formation at the edge of the joints (Figure 1). Despite significant social demand for more information, risk factors of this disease identified by epidemiologic studies have to date been limited to age, obesity, trauma history, occupation, and gender [1]. These factors are closely related to the accumulation of mechanical stress to joints.

In an effort to clarify the mechanisms whereby the mechanical stress leads to OA development, experimental animal models in which joint instability is induced through surgical intervention have been developed in dogs, rabbits, guinea pigs and rats [2-8]. Considering that mouse is now the most ideal animal for the molecular study due to recent progress in mouse genetics and the availability of transgenic and knockout mice, we and others have established mechanical instability-induced OA models in mice that are reproducible and resemble human OA, using a microsurgical technique to produce instability in the knee joints. Most popular is the model developed by Glasson’s group which involves destabilization of the medial meniscus (DMM) by transection of meniscotibial ligament anchoring the medial meniscus to the tibial plateau (9). We have developed the medial model by resection of the medial meniscus and medial collateral ligament [10]. In addition, there is a traditional anterior cruciate ligament transection (ACLT) model that has also been used in larger animals [11].

Proteinases for OA induction

Normal joint cartilage is constituted of a framework of type II collagen (COL2), in which proteoglycan connected to hyaluronic acid waves smoothly. Because it contains a large quantity of water, proteoglycan provides elasticity and lubricity to the joint surface. However, in the OA cartilage, most of the proteoglycans are cut, fragmented, and floating in the synovial fluid. Due
OA development. Our previous report showed that levels of TNF-\(\alpha\), IL-1, IL-6 as well as fibroblast growth factor-2 in the synovial fluid from knee joints of OA patients were much lower than those of rheumatoid arthritis patients [20]. Furthermore, a previous report using a mouse ACLT model showed that mice lacking IL-1, IL-1-converting enzyme, stromelysin 1 or inducible nitric oxide synthase unexpectedly exhibited an acceleration of cartilage degradation, implying that these pro-inflammatory factors do not stimulate, but rather inhibit such degradation [11].

PGE2, a representative pro-inflammatory factor, is also produced more abundantly in the OA cartilage than in normal cartilage [21], and microsomal PGE synthase-1 (mPGES-1) is a terminal enzyme for the PGE2 production in chondrocytes of OA patients [22]. Although we created the medial OA model in the PGES-1 knockout mice, the cartilage degradation and osteophyte formation were comparable to the wild-type littermates [23]. We therefore believe that inflammation may be associated with the OA process as a consequence, but might not have a central role in the cause of OA initiation or progression.

Pro-inflammatory factors and OA

Besides these matrix proteins, pro-inflammatory factors like prostaglandins (PGs), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-1 (IL-1), IL-6, and nitric oxides are reported to induce proteases through their respective receptors [19]. These pro-inflammatory factors are also reported to be produced by synovial cells under the stimulation of the undegraded or fragment-ed matrix proteins [17]. However, it is suspected that these cytokines play significant roles in the OA development. Our previous report showed that levels of TNF-\(\alpha\), IL-1, IL-6 as well as fibroblast growth factor-2 in the synovial fluid from knee joints of OA patients were much lower than those of rheumatoid arthritis patients [20]. Furthermore, a previous report using a mouse ACLT model showed that mice lacking IL-1, IL-1-converting enzyme, stromelysin 1 or inducible nitric oxide synthase unexpectedly exhibited an acceleration of cartilage degradation, implying that these pro-inflammatory factors do not stimulate, but rather inhibit such degradation [11].

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Chondrocyte hypertrophy and OA

Our examination of the time course of histology of the mouse joint cartilage using the medial OA model revealed that type X collagen (COL10) and MMP-13 were significantly induced during OA progression (Figure 2) [10,24]. COL10 expression, a specific marker of hypertrophic chondrocytes, appeared in the superficial and middle zones above the tidemark at 4 weeks, consistent with previous studies [25,26]. MMP-13 expression appeared in the hypertrophic chondrocytes.
above the tidemark at 8 weeks. These findings suggest that articular chondrocytes undergo hypertrophic differentiation in response to joint instability, and the hypertrophic chondrocytes express MMP-13 that may degrade the cartilage matrix.

Since a transcriptional activator Runx2 has been known to induce both chondrocyte hypertrophy and MMP-13 expression [27-29], we then examined the involvement of Runx2 during OA development [24]. Runx2 expression was induced above the tidemark in the cartilage as early as 2 weeks, enhanced at 4 weeks, and decreased thereafter by the OA induction, which was not observed in the sham-operated cartilage (Figure 3).

For the functional analyses of Runx2, we used heterozygous Runx2-knockout mice (Runx2+/–), since homozygous Runx2-knockout (Runx2–/–) mice died just after birth. The Runx2+/– mice showed normal skeletal development and articular cartilage under physiological conditions. Both COL10 and MMP-13 expressions were decreased by the Runx2 insufficiency (data not shown), indicating that chondrocyte hypertrophy and MMP-13 induction during OA progression is at least partly mediated by Runx2.

When the OA progression was compared between WT and Runx2+/– joints, the cartilage degradation in Runx2+/– was much milder than that of the WT cartilage at 8 weeks and thereafter (Figure 3A). The Runx2+/– joint also showed decreased osteophyte formation (Figure 3B). These findings demonstrate that Runx2 contributes to cartilage degradation and the subsequent osteophyte formation under joint instability.

Our differential display analysis identified a novel molecule that was up-regulated by a high phosphate diet in association with calcification of auricular cartilage in mice, and we called it carminerin [30,31]. The knockout (carminerin –/–) mice did not show skeletal abnormality under physiological conditions. When we created the ACLT OA model, the joint destruction normally occurred; however, osteophyte formation seemed to be decreased by the carminerin knockout, indicating that carminerin is not essential for cartilage degradation but plays a role in the osteophyte formation (Figure 4) [32].

Chondrocyte apoptosis and OA

In addition to hypertrophic differentiation of chondrocytes, chondrocyte apoptosis is known to be involved in OA development [33]. Intraarticular injection of a pan-caspase inhibitor has been reported to suppress cartilage degradation under OA induction in a mouse ACLT model [34]. When we created the OA medial model in the hetero knockout mice of osteoprotegerin that is well known as a decoy receptor of RANKL for osteoclastic bone resorption, joint destruction was enhanced as compared to the wild-type littermates [35]. On the contrary, an intraarticular injection of recombinant osteoprotegerin suppressed joint destruction with a decrease of apoptotic chondrocytes. Since tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a ligand of osteoprotegerin, induces chondrocyte apoptosis [36,37], osteoprotegerin might inhibit the apoptosis induced by TRAIL. These in vivo findings clearly demonstrate that,
not only chondrocyte hypertrophy but also chondrocyte apoptosis, both of which are signals of endochondral ossification, play some roles in cartilage degradation during OA development under mechanical stress.

**Conclusion**

Figure 5 summarizes our hypothesis of the molecular background of OA progression under mechanical stress in joints. As the mechanism involved in this stress causing protease production, in addition to matrix proteins and pro-inflammatory signals, we hereby propose the importance of chondrocyte hypertrophy and apoptosis, which are signals for endochondral ossification. The proteinases produced by hypertrophic chondrocytes cause cartilage degradation at the center of the joint and osteophyte formation at the periphery. The difference of the two sites may depend on the vascularity. At the periphery, vascularity is accessible from synovium or tendon, which causes endochondral ossification to occur and make osteophytes, just like at the growth plate. Carminerin may play a role in the chondrocyte calcification at this stage. However, in the center, the vascularity is not accessible from the edge, so that it ends up with cartilage degradation without being replaced by bone. The ultimate aim of our study is to identify the molecular targets for clinical treatment of OA. Although we primarily used mouse genetics approaches, we have attempted to confirm the reproducibility of the mouse findings in humans as well through human gene polymorphism or clinical biochemical studies. Among the molecules introduced in this review, there are some whose suppression ameliorated skeletal disorders under pathological conditions but did not affect physiological conditions, indicating that the treatment targeting these molecules may lead to an ideal treatment without side effects on physiological functions. In fact, clinical trials based on the present findings are currently being practically planned.
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Figure 4: Von Kossa staining and three-dimensional computed tomography (3D-CT) of knee joints of carminerin–/– and wild-type (WT) littermates in the ACL T model; OA was induced at the posterior tibias of the knee joint of 8-week-old mice and examined 10 weeks after surgery.

Figure 5: Molecular background of OA progression under mechanical stress by mouse genetics approaches.

References


