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REVIEW ARTICLE

Osteoarthritis: toward a comprehensive understanding of pathological mechanism

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Osteoarthritis (OA) is the most common degenerative joint disease and a major cause of pain and disability in adult individuals. The etiology of OA includes joint injury, obesity, aging, and heredity. However, the detailed molecular mechanisms of OA initiation and progression remain poorly understood and, currently, there are no interventions available to restore degraded cartilage or decelerate disease progression. The diarthrodial joint is a complicated organ and its function is to bear weight, perform physical activity and exhibit a joint-specific range of motion during movement. During OA development, the entire joint organ is affected, including articular cartilage, subchondral bone, synovial tissue and meniscus. A full understanding of the pathological mechanism of OA development relies on the discovery of the interplaying mechanisms among different OA symptoms, including articular cartilage degradation, osteophyte formation, subchondral sclerosis and synovial hyperplasia, and the signaling pathway(s) controlling these pathological processes.

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INTRODUCTION

Osteoarthritis (OA) is the most common degenerative joint disease, affecting more than 25% of the population over 18 years-old. Pathological changes seen in OA joints include progressive loss and destruction of articular cartilage, thickening of the subchondral bone, formation of osteophytes, variable degrees of inflammation of the synovium, degeneration of ligaments and menisci of the knee and hypertrophy of the joint capsule.¹ The etiology of OA is multi-factorial and includes joint injury, obesity, aging, and heredity.^{1–5} Because the molecular mechanisms involved in OA initiation and progression remain poorly understood, there are no current interventions to restore degraded cartilage or decelerate disease progression. Studies using genetic mouse models suggest that growth factors, including transforming growth factor- β (TGF- β), Wnt3a and Indian hedgehog, and signaling molecules, such as Smad3, β -catenin and HIF-2 α ,^{6–10} are involved in OA development. One feature common to several OA animal models is the upregulation of Runx2.^{7–8,11–13} Runx2 is a key

transcription factor directly regulating the transcription of genes encoding matrix degradation enzymes in articular chondrocytes.^{14–17} In this review article, we will discuss the etiology of OA, the available mouse models for OA research and current techniques used in OA studies. In addition, we will also summarize the recent progress on elucidating the molecular mechanisms of OA pain. Our goal is to provide readers a comprehensive coverage on OA research approaches and the most up-to-date progress on understanding the molecular mechanism of OA development.

ETIOLOGY

OA is the most prevalent joint disease associated with pain and disability. It has been forecast that 25% of the adult population, or more than 50 million people in the US, will be affected by this disease by the year 2020 and that OA will be a major cause of morbidity and physical limitation among individuals over the age of 40.^{18–19} Major clinical symptoms include chronic pain, joint instability, stiffness and

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radiographic joint space narrowing.²⁰ Although OA primarily affects the elderly, sports-related traumatic injuries at all ages can lead to post-traumatic OA. Currently, apart from pain management and end stage surgical intervention, there are no effective therapeutic treatments for OA. Thus, there is an unmet clinical need for studies of the etiology and alternative treatments for OA. In recent years, studies using the surgically induced destabilization of the medial meniscus (DMM) model and tissue or cells from human patients demonstrated that genetic, mechanical, and environmental factors are associated with the development of OA. At the cellular and molecular level, OA is characterized by the alteration of the healthy homeostatic state toward a catabolic state.

Aging

One of the most common risk factors for OA is age. A majority of people over the age of 65 were diagnosed with radiographic changes in one or more joints.^{21–25} In addition to cartilage, aging affects other joint tissues, including synovium, subchondral bone and muscle, which is thought to contribute to changes in joint loading. Studies using articular chondrocytes and other cells suggest that aging cells show elevated oxidative stress that promotes cell senescence and alters mitochondrial function.^{26–29} In a rare form of OA, Kashin-Back disease, disease progression was associated with mitochondrial dysfunction and cell death.³⁰ Another hallmark of aging chondrocytes is reduced repair response, partially due to alteration of the receptor expression pattern. In chondrocytes from aged and OA cartilage, the ratio of TGF- β receptor ALK1 to ALK5 was increased, leading to down-regulation of the TGF- β pathway and shift from matrix synthesis activity to catabolic matrix metalloproteinase (MMP) expression.^{31–32} Recent studies also indicate that methylation of the entire genomic DNA displayed a different signature pattern in aging cells.^{33–34} Genome-wide sequencing of OA patients also confirmed that this epigenetic alteration occurred in OA chondrocytes,^{35–37} partially due to changes in expression of Dnmts (methylation) and Tets (de-methylation) enzymes.^{38–40}

Obesity

In recent years, obesity has become a worldwide epidemic characterized by an increased body composition of adipose tissue. The association between obesity and OA has long been recognized.^{41–42} Patients with obesity develop OA earlier and have more severe symptoms, higher risk for infection and more technical difficulties for total joint replacement surgery. In addition to increased biomechanical loading on the knee joint, obesity is thought to contribute to low-grade systemic inflammation through

secretion of adipose tissue-derived cytokines, called adipokines.^{43–45} Specifically, levels of pro-inflammatory cytokines, including interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor alpha (TNF- α) were elevated^{46–50} in high-fat diet-induced mouse obesity models^{51–54} and in obese patients.^{55–57} These inflammatory factors may trigger the nuclear factor- κ B (NF- κ B) signaling pathway to stimulate an articular chondrocyte catabolic process and lead to extracellular matrix (ECM) degradation through the up-regulation of MMPs.^{58–60}

Sport injury

Knee injury is the major cause of OA in young adults, increasing the risk for OA more than four times. Recent clinical reports showed that 41%–51% of participants with previous knee injuries have radiographic signs of knee OA in later years.⁶¹ Cartilage tissue tear, joint dislocation and ligament strains and tears are the most common injuries seen clinically that may lead to OA. Trauma-related sport injuries can cause bone, cartilage, ligament, and meniscus damage, all of which can negatively affect joint stabilization.^{62–66} Signs of inflammation observed in both patients with traumatic knee OA and in mouse injury models include increased cytokine and chemokine production, synovial tissue expansion, inflammatory cell infiltration, and NF- κ B pathway activation.⁶⁷

Inflammation

It has been established that the chronic low-grade inflammation found in OA contributes to disease development and progression. During OA progression, the entire synovial joint, including cartilage, subchondral bone, and synovium, are involved in the inflammation process.⁶⁸ In aging and diabetic patients, conventional inflammatory factors, such as IL-1 β and TNF- α , as well as chemokines, were reported to contribute to the systemic inflammation that leads to activation of NF- κ B signaling in both synovial cells and chondrocytes. Innate inflammatory signals were also involved in OA pathogenesis, including damage associated molecular patterns (DAMPs), alarmins (S100A8 and S100A9) and complement.^{69–71} DAMPs and alarmins were reported to be abundant in OA joints, signaling through either toll-like receptors (TLR) or the canonical NF- κ B pathway to modulate the expression of MMPs and a disintegrin and metalloprotease with thrombospondin motif (ADAMTS) in chondrocytes.^{72–76} Complement can be activated in OA chondrocytes and synovial cells by DAMPs, ECM fragments and dead-cell debris.^{77–78} Recent studies further clarified that systemic inflammation can re-program chondrocytes through inflammatory mediators toward hypertrophic differentiation and catabolic responses through the NF- κ B pathway,^{9–10,79} the ZIP8/Zn⁺/

MTF1 axis,⁸⁰ and autophagy mechanisms.^{81–85} Indeed, the recent Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of OA and control samples provide evidence that inflammation signals contribute to OA pathogenesis through cytokine-induced mitogen-activated protein (MAP) kinases, NF- κ B activation, and oxidative phosphorylation.⁸⁶

Genetic predisposition

An inherited predisposition to OA has been known for many years from family-based studies.^{87–89} Although the genetics of OA are complex, the genetic contribution to OA is highly significant. Over the past decade, the roles of genes and signaling pathways in OA pathogenesis have been demonstrated by *ex vivo* studies using tissues derived from OA patients and *in vivo* studies using surgically induced OA animal models and genetic mouse models. For example, alterations in TGF- β , Wnt/ β -catenin, Indian Hedgehog (Ihh), Notch and fibroblast growth factor (FGF) pathways have been shown to contribute to OA development and progression by primarily inducing catabolic responses in chondrocytes.^{8,90–95} Such responses converge on *Hif2a*, *Runx2*, and inflammatory mediators that lead to cartilage ECM degradation through the increased expression of MMPs and ADAMTS activity.^{80,96–99} Recent studies of genome-wide association screens (GWAS) that have been performed on large numbers of OA and control populations throughout the world have confirmed over 80 gene mutations or single-nucleotide polymorphisms (SNPs) involved in OA pathogenesis. Some of the genes are important structural and ECM-related factors (*Col2a1*, *Col9a1*, and *Col11a1*), and critical signaling molecules in the Wnt (*Sfrp3*), bone morphogenetic protein (BMP) (*Gdf5*), and TGF- β (*Smad3*) signaling pathways; most of these genes have been previously implicated in OA or articular cartilage and joint maintenance by studies using mouse models of induced genetic alteration- or surgically induced OA.^{100–106} A recent arcOGEN Consortium genome-wide screen study¹⁰⁷ identified new SNPs in several genes, including *GNL3*, *ASTN2*, and *CHST11*. These findings need to be verified by further studies.

MOUSE MODELS FOR OA RESEARCH

DMM model

DMM was developed 10 years ago and is a well established surgical OA model in mice and rats. It is widely used to study OA initiation and progression in combination with transgenic mouse models and aging and obesity models. DMM surgery was performed by transection of the medial meniscotibial ligament (MMTL),^{26–27} Briefly, following the initial incision, the joint capsule on the medial side was

incised using scissors to expose either the intercondylar region or the MMTL, which anchors the medial meniscus (MM) to the tibial plateau. The MMTL was visualized under a dissection microscope and the MMTL was cut using microsurgical scissors, releasing the ligament from the tibia plateau thus destabilizing the medial meniscus. Closure of the joint capsule and skin was with a continuous 8–0 tapered Vicryl suture. As a control for DMM studies, sham surgery was performed by only exposing the medial side of knee joint capsule. Because of the medial displacement of the meniscus tissue, greater stress occurred on the posterior femur and central tibia, especially on the medial side.¹⁰⁸ Histology demonstrated the severity of OA lesions at 4-weeks post-surgery with fibrillation of the cartilage surface. Cartilage destruction and subchondral bone sclerosis developed 8 weeks post-surgery and osteophyte formation was seen 12-weeks post- surgery.^{98,109–111}

Aging model

As a degenerative disease, OA always occurs in elderly populations; thus, aging is a major risk factor for the most common form in humans, spontaneous OA. Several laboratory animals develop spontaneous OA, which approximates the stages of human OA progression. These animal models are valuable tools for studying natural OA pathogenesis.^{112–113} The most commonly used inbred strain of laboratory mouse is C57/BL6; these mice usually develop knee OA at about 17 months of age.¹¹² The STR/ort mouse is one strain that easily develops spontaneous OA. It requires 12–20 weeks for STR/ort mice to develop articular cartilage destruction.^{114–116} This may be partially due to their heavier body weight compared with other mouse strains. Given the background genetic consistency, although aging OA models have many advantages, it normally requires at least one year for mice to model the disease. Therefore, surgically induced OA models^{107,117} and genetic mouse models are preferred in recent decades for their relatively fast induction for use as aging models for the study of OA lesions.

In addition to the mouse, the Dunkin Hartley guinea pig provides an aging model widely used to study OA development.¹¹⁸ The Dunkin Hartley guinea pig can develop a spontaneous, age-related OA phenotype within 3 months. The severity of OA lesions increases with age, and moderate to severe OA is observable in 18-month-old animals. Histological analysis demonstrated that the spontaneous OA progression in Dunkin Hartley guinea pig resembles that of humans. Thus, the Dunkin Hartley guinea pig is a useful animal to study the pathogenesis and evaluation of potential treatments for human OA.

Obesity model

It has become evident that obesity contributes to a variety of musculoskeletal diseases, particularly OA, because of inflammatory and metabolic responses.¹¹⁹ Together with surgically induced injury and genetic models, mouse obesity models are widely used to explore the mechanisms of obesity-induced OA. The obese mouse model is induced by a high-fat diet, in which 60% of calories are derived from fat as opposed to the normal 13%.¹²⁰ The entire joint tissue, but especially synovium tissue, is affected by the high-fat diet. A synovial inflammation phenotype has been independently reported by different laboratories.⁵⁴ An elevated systemic inflammation was observed in obese mice following DMM surgery. Serum levels of pro-inflammatory factors, including interleukin-12p70,⁵⁴ interleukin-6, TNF α and several other chemokines, were increased, suggesting a role for obesity in the development of post-traumatic OA (PTOA).

Genetic mouse models

Genetic mouse models have recently become widely used to investigate the cellular and molecular mechanisms of OA development. Based on the GWAS studies of human patients, mutant mouse strains were generated carrying either mutant genes or SNPs. For example, *Del1*^{+/-} mice carried a mutation in the collagen II gene. Both *Del1*^{+/-} mice and *Col9a1*^{-/-} mice developed spontaneous OA.¹²¹ Because cartilage functions as a skeletal architect, conventional gene deletion approaches have the drawback of causing embryonic lethality or severe skeletal deformation. To overcome embryonic lethality and bypass the limits of constitutive gene knockout (KO), inducible conditional KO technology has been widely used. This usually combines Cre-loxP gene targeting with tamoxifen-induced nuclear translocation of CreER fusion protein driven by tissue-specific promoters. The *Col2a1*-Cre^{ERT2}, *Agc1*-Cre^{ERT2} and *Prg4*-Cre^{ERT2} transgenic mice¹²²⁻¹²⁴ have become powerful tools for targeting joint tissue to study the mechanism of OA development. Based on the gene expression pattern, both *Col2a1* and *Agc1* can efficiently target chondrocytes in the growth plate cartilage, articular cartilage and temporomandibular joint. Because *Agc1* is expressed more robustly than *Col2a1* in adult cartilage tissue, *Agc1* is expected to better target chondrocytes in adult mice.¹²³ In addition to chondrocytes, *Agc1* were also reported to target nucleus pulposus tissue in the intervertebral disc.¹²³ *Prg4* only targets the superficial layer of articular chondrocytes.¹²⁴ It needs to be emphasized that all of these genetic tools are used to address the importance of cartilage tissue in OA development. Additional CreER transgenic mice need to be developed to

Table 1. Available transgenic mouse models for osteoarthritis research

Gene	Targeting tissue	Pathway
<i>Del1</i> ¹²⁵	Global	ECM
<i>Col9a1</i> ¹²⁶	Global	ECM
<i>Tgfb2</i> ⁹⁰	4xMRE	TGF- β
<i>Smad3</i> ⁶	Global	TGF- β
<i>Smurf2</i> ¹²⁷	Col2-Cre	TGF- β
<i>Tgfb2</i> ⁹¹	Col2-CreER	TGF- β
<i>Frzb</i> ¹²⁸	EllCre	Wnt
<i>β-catenin</i> ⁷	Col2-CreER	Wnt
<i>Rbpjk</i> ⁹³	Prx1Cre	Notch
<i>Fgfr1</i> ¹²⁹	Col2-CreER	FGF
<i>Smo</i> ⁸	Col2-Cre	Ihh
<i>Runx2</i> ¹¹	Global	
<i>Hif2a</i> ⁹	Global	
<i>Mmp13</i> ⁹⁷	Global	
<i>Mmp13</i> ¹³⁰	Col2-CreER	
<i>Adams5</i> ⁹⁸	Global	

Abbreviations: ECM, extracellular matrix; FGF, fibroblast growth factor; Ihh, Indian Hedgehog; TGF- β , transforming growth factor- β .

efficiently target subchondral bone, synovial tissue and meniscus.

Using these transgenic mice, specific genes have been studied in chondrocyte-specific experiments to dissect their role in OA. *In vivo* studies employing mutant mice suggest that pathways involving (i) receptor ligands, such as TGF- β 1, Wnt3a, and Indian hedgehog, (ii) signaling molecules, such as Smads, β -catenin, Runx2 and HIF-2 α and, (iii) peptidases, such as MMP13 and ADAMTS4/5, have some degree of involvement in OA development. Table 1 summarizes the mutant lines available for OA study.

TGF- β and its downstream molecules have important roles in OA pathogenesis. Mutations of *Smad3*, a central molecule in TGF- β signaling, have been found in patients with early-onset OA.¹³¹⁻¹³³ It has been known for years that TGF- β promotes mesenchymal progenitor cell differentiation and matrix protein synthesis and inhibits chondrocyte hypertrophy. TGF- β signaling may play differential roles in joint tissues during OA development. For example, global deletion of *Smad3* causes chondrocyte hypertrophy and OA-like articular cartilage damage.⁶ The deletion of *Tgfb2*, encoding for type II TGF- β receptor,⁹¹ or *Smad3*¹² in articular chondrocytes also led to an OA-like phenotype. In contrast, the activation of TGF- β signaling in mesenchymal progenitor cells of subchondral bone also caused OA-like lesions.¹³⁴ These findings suggest that TGF- β signaling may have differential roles in various joint tissues¹³⁵ and that therapeutic interventions targeting TGF- β signaling may require a tissue-specific approach.

TECHNIQUES FOR OA STUDIES

In vitro studies

***In vitro* articular chondrocyte isolation and culture.** To investigate signaling mechanisms in articular cartilage, primary human articular chondrocytes will be obtained from surgically discarded cartilage tissues. Briefly, full-thickness sections of cartilage are excised from the subchondral bone. The cartilage pieces will be digested for about 15 h using a digestion buffer. The isolated cells will be then collected and filtered to remove undigested tissue and debris, and washed with Hanks' buffered salt solution. The cells will be then re-suspended in chondrocyte basal medium and plated in high density monolayer cultures as shown in Table 2.^{136–137} Human articular chondrocytes can also be cultured in three dimensions. Briefly, 4×10^6 freshly isolated human articular chondrocytes will be re-suspended in alginate solution and the cell suspension is added drop-wise into $102 \text{ mmol} \cdot \text{L}^{-1}$ CaCl_2 to form beads. After washing the beads with $0.15 \text{ mol} \cdot \text{L}^{-1}$ NaCl and basal medium, the chondrocytes encapsulated in alginate beads will be cultured in three dimensions with basal medium.^{138–139}

***In vitro* human articular cartilage explant culture.** Osteochondral tissues from radiographically and anatomically normal joints will be obtained from patients with different surgeries, such as oncologic surgical procedures, meniscal tear repair or total knee joint replacement. The collected osteochondral tissues will be first washed with sterile phosphate-buffered saline (PBS). Fresh cartilage samples will be harvested from the femoral condyle using a 6 mm diameter biopunch. The cartilage explants will be cultured in chondrocyte basal medium.¹⁴⁰

Histology/histomorphometry

Knee cartilage samples to be used for histological and histomorphometric analyses will be fixed in 10% neutral buffered formalin (NBF), decalcified in 14% EDTA for 10 days and embedded in paraffin. The paraffin-embedded samples will be cut into $5 \mu\text{m}$ sections and stained with Alcian blue/Hematoxylin-Orange G (ABH) or Safranin O/Fast green to determine changes in architectures of cartilage, bone, and synovial tissues throughout OA progression. Quantitative histomorphometric analyses of

ABH-stained sections can be performed using a Visiopharm analysis system.¹⁴¹ Using this system, high resolution digital images of histology slides can be obtained. Cartilage thickness will be measured from the middle of the femoral and tibial condyles. Cartilage area will be traced from both articular cartilage surfaces. The tidemark will be used to delineate the upper and deep zone of articular cartilage.^{91,93}

OARSI score system

Several scoring systems have been developed to semi-quantify the severity of OA lesions of the knee. A scoring system recommended by the Osteoarthritis Research Society International (OARSI) society is based on continuous histological staining of the knee joint. A 0–6 subjective scoring system, as shown in Table 3, is applied to all four quadrants through multiple step sections of the joint. Sagittal sections obtained every $80 \mu\text{m}$ across the medial femoral-tibial joint will be used to determine the maximal and cumulative scores.¹⁴²

Nanoindentation

It is necessary to understand changes in mechanical properties of OA cartilage across multiple length scales because they directly reflect cartilage functional changes during degradation.¹⁴³ Atomic force microscopy (AFM)-based nanoindentation is well-suited for evaluating changes at a nm-to- μm scale that is comparable to the sizes of matrix molecules and cells.¹⁴⁴ For AFM-nanoindentation measurement, a microspherical or a pyramidal tip is programmed to indent the sample tissues, cells or tissue sections to a pre-set force or depth. An effective indentation modulus can be calculated by fitting the loading portion of each indentation force versus depth curve to the elastic Hertz model.¹⁴⁵ The use of nanoindentation over the past decade has uncovered many new aspects of cartilage structure-mechanics relationships and OA pathomechanics. Highlights among these include micromechanical anisotropy and heterogeneity of healthy and OA cartilage¹⁴⁶ or meniscus,¹⁴⁷ cartilage weakening in spontaneous^{148–149} and post-traumatic^{150–152} OA, mechanics of individual chondrocytes,^{151,153} and quality evaluation of engineered neo-tissues.^{154–156}

Notably, AFM-nanoindentation has made it possible to study the mechanical properties of murine cartilage. Previously, the $\sim 100 \mu\text{m}$ thickness of murine cartilage prevented such attempts. Because *in vivo* OA studies are largely dependent on murine models,¹⁵⁷ nanoindentation provides a critical bridge across two crucial fields of OA research: biology and biomechanics. The benefit of nanoindentation for murine model studies has been demonstrated by a number of recent studies. For example,

Table 2. Monolayer culture conditions for human primary articular chondrocytes

Plate type	Volume per well	No. of cells per well
6-well	2.5 mL	1×10^6
12-well	1 mL	4×10^5
24-well	0.5 mL	2×10^5

Table 3. The recommended semi-quantitative scoring system¹⁴³

Grade	Osteoarthritic damage
0	Normal
0.5	Loss of Safranin O without structural changes
1	Small fibrillations without loss of cartilage
2	Vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina
3	Vertical clefts/erosion to the calcified cartilage extending to < 25% of the articular surface
4	Vertical clefts/erosion to the calcified cartilage extending to 25%–50% of the articular surface
5	Vertical clefts/erosion to the calcified cartilage extending to 50%–75% of the articular surface
6	Vertical clefts/erosion to the calcified cartilage extending to > 75% of the articular surface

cartilage in mice lacking collagen IX (*Col9a1*^{-/-})¹⁴⁸ showed abnormally higher moduli, while those lacking lubricin (*Prg4*^{-/-})¹⁵⁸ or chondroadherin (*Chad*^{-/-})¹⁵⁹ showed lower moduli. *Col9a1*^{-/-} and *Prg4*^{-/-} mice also developed macroscopic signs of OA,^{148,158} underscoring the high correlation between abnormalities in cartilage biomechanics and OA. Li *et al.* also recently demonstrated the applicability of nanoindentation to the murine meniscus.¹⁶⁰ Further applications of nanoindentation to clinically relevant OA models, such as the DMM model,¹¹⁰ hold the potential of assessing OA as an entire joint disease through biomechanical symptoms in multiple murine synovial tissues.

Two other recent technological advances provide paths to further in-depth studies. First, Wilusz *et al.*¹⁶¹ stained cartilage cryosections with immunofluorescence antibodies of the pericellular matrix signature molecules, type VI collagen and perlecan.¹⁶² Using immunofluorescence guidance, nanoindentation was used to delineate the mechanical behavior of cartilage pericellular matrix and ECM,^{161–163} and to reveal the role of type VI collagen in each matrix by employing *Col6*^{-/-} mice.¹⁶⁴ Therefore, it is now possible to directly examine the relationships across micro-domains between biochemical content and biomechanical properties of cartilage,¹⁶¹ meniscus¹⁶⁵ or other synovial tissues *in situ*. Second, Nia *et al.*¹⁶⁶ converted the AFM to a high-bandwidth nanorheometer. This tool enabled separation of the fluid flow-driven poroelasticity and macromolecular frictional intrinsic viscoelasticity that govern cartilage energy-dissipative mechanics.^{166–168} Hydraulic permeability, the property that regulates poroelasticity, was found to be mainly determined by aggrecan rather than collagen¹⁶⁹ and to change more drastically than modulus upon depletion of aggrecan.^{166,170} This new tool provides a comprehensive approach beyond the scope of elastic modulus for assessing cartilage functional changes in OA.

MOLECULES MEDIATING OA PAIN

The perception of OA pain is a complex and dynamic process involving structural and biochemical alterations at

the joint as well as in the peripheral and central nervous systems. While there have been extensive studies of mediators of OA joint degeneration, only recently have studies begun to characterize biochemical influences on and in the peripheral and central nervous systems in OA. In this regard, OA appears to show similarities and differences with other conditions causing pain.^{171–172} There are a wide variety of signaling pathways linked to joint destruction and/or pain. In this section we will discuss three emerging and highly relevant pathways that provide insight into the mechanisms underlying OA pain.

Chemotactic cytokine ligand 2/chemokine (C–C motif) receptor 2

Chemotactic cytokine ligand 2 (CCL2), also known as monocyte chemoattractant protein 1 (MCP-1), is well-known to mediate the migration and infiltration of monocytes and macrophages by signaling through chemokine (C–C motif) receptor 2 (CCR2).¹⁷³ In arthritis, CCL2 promotes inflammation of the joint.¹⁷⁴ Evidence also suggests that CCL2 is an important mediator of neuroinflammation.^{175–176} In neuropathic pain, CCL2 expression is increased in microglia and in sensory neurons in the dorsal root ganglia (DRGs), where CCL2 can be further transported and released into central spinal nerve terminals. Increased CCL2/CCR2 signaling has been correlated with direct excitability of nociceptive neurons and microglial activation, leading to persistent hyperalgesia and allodynia.^{177–178}

In a DMM mouse OA model, CCL2 and CCR2 levels were elevated in DRGs at 8 weeks post surgery, correlating with increased OA-associated pain behaviors. Increased CCL2 and CCR2 levels in the DRG were thought to mediate pronociceptive effects both by increasing sensory neuron excitability through CCL2/CCR2 signaling directly in DRG sensory neurons and through CCL2/CCR2-mediated recruitment of macrophages in the DRG. Compared with wild-type mice, *Ccr2*-null mice showed reduced pain behaviors following DMM with similar levels of joint damage.¹⁷⁹ Although CCR2 antagonists are currently being assessed in clinical studies, no clinical studies have targeted CCL2 or CCR2 in OA pain.¹⁸⁰

Nerve growth factor/tropomyosin receptor kinase A

In both clinical and animal studies, the targeted inhibition of nerve growth factor (NGF) and inhibition of its cognate receptor, tropomyosin receptor kinase A (TrkA), reduced OA pain. Clinically, the systemic administration of NGF caused persistent whole-body muscle hyperalgesia in healthy human subjects,^{174,177} while anti-NGF antibody, tanezumab, therapy significantly reduced OA pain.^{181–184} There are a number of potential mechanisms through which NGF mediates pain. Over-expressed NGF in peripheral tissues can bind directly to TrkA at sensory neuron nerve terminals and be retrogradely transported to the DRG. There it stimulates sensory neurons to activate mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling.¹⁸⁵ The activation of the NGF-MAPK/ERK axis upregulates the expression of pain-related molecules, including transient receptor potential cation channel subfamily V member 1 (TRPV1), substance P, calcitonin gene-related peptide (CGRP), brain-derived neurotrophic factor (BDNF), and nociceptor-specific ion channels, such as Ca_v 3.2, 3.3, and Na_v1.8.^{186–188}

In addition to direct signaling of sensory neurons, NGF promotes algescic effects by targeting other cell types. For example, NGF/TrkA signaling occurs in mast cells, triggering release of pro-inflammatory and pain mediators, including histamine and prostaglandins, in addition to NGF.^{186,189} NGF signaling is upregulated by pro-inflammatory mediators, and NGF promotes leukocyte chemotaxis and vascular permeability, further stimulating inflammation.^{190–192} NGF/TrkA signaling further promotes angiogenesis and nerve growth. The process of angiogenesis is not only inflammatory, but also serves as a track for nerve growth into the joint.¹⁹³

Given the high efficacy of targeting NGF in a clinical study on reducing OA pain, it is of great interest to further define NGF/TrkA pain signaling mechanisms and to find additional therapeutic targets in this pathway. Recent evidence indicates that loss of PKC δ signaling significantly increases both NGF and TrkA in the DRG and synovium, is associated with increased MAPK/ERK signaling at the innervating DRGs, and is associated with OA hyperalgesia.¹⁹⁴ However, in recent clinical studies, a small population of patients treated with systemic anti-NGF therapy exhibited rapid progression of OA and were more prone to bone fractures.¹⁹⁵ Considering the analgesic effects by anti-NGF therapy on OA-associated pain, understanding of the precise roles of the NGF/TrkA pathway in different joint tissues in OA and OA-associated pain is of great interest.

ADAMTS5

The use of *Adamts5* KO mice and therapeutic treatment with anti-ADAMTS5 antibody in wild-type mice produce

inhibition of ADAMTS5 signaling/expression in the DMM model, resulting in reduction of both joint degeneration and pain.^{98,196–197} ADAMTS5 is a major aggrecanase, and because aggrecan is a major component of the proteoglycans in cartilage that provides compressive resistance, ADAMTS5 is thought to be a critical mediator of cartilage degeneration during the development of OA.¹⁹⁸ Although variations in pain signaling can be independent from the degree of joint degeneration, the use of *Adamts5* KO mice and direct inhibition of joint degeneration with anti-ADAMTS5 antibody may provide insight into how joint degeneration produces OA pain. For example, hyaluronan fragments generated by ADAMTS5 have been suggested to directly stimulate nociceptive neurons as well as glial activation, promoting increased pain perception.^{196,199} Furthermore, inhibition of ADAMTS5 following DMM resulted in reduced levels of CCL2 in DRG neurons, thus suggesting a role for CCL2 in OA-specific pain.¹⁹⁷

Pain-related behavior tests

Pain is the most common reason patients seek medical treatment and is a major indication for joint replacement surgery.^{200–201} Therefore, evaluating pain in pre-clinical animal models is of critical importance to better understand mechanisms of and to develop treatments for OA pain. The evaluation of OA pain in animals involves indirect and direct measures.

Recognizing pain as a clinical sign and quantitatively assessing pain intensity are essential in research for effective OA pain management. Rodent animal models are routinely used for basic and pre-clinical studies because of the relatively low cost of animal maintenance, the abundance of historical data for comparison, and smaller amounts of drugs required for experimental studies. For pain measurements, rodents have advantages over other small animal models, such as rabbits, which present challenges to obtain a pain response and are immobile if startled by an unfamiliar observer. Mice are usually used for the development of genetically engineered strains to enable molecular understanding of OA progression and pain *in vivo*.²⁰² Larger animals, including dogs, sheep, goats, and horses are also sometimes used for modeling OA pain.^{202–203}

A wide range of direct and indirect measures of pain are used in small animal models of OA. Indirect and/or direct measures of pain include static or dynamic weight bearing, foot posture, gait analysis, spontaneous activity, as well as sensitivity to mechanical allodynia, mechanical hyperalgesia, and thermal, and cold stimuli.^{202–203} Among indirect tests involving pain-evoked behaviors, mechanical stimuli may be the most correlated with OA pain. A commonly used measure of indirect pain is the von Frey

test for mechanical allodynia using filaments to assess referred pain.^{186,194,196,202,204} Direct mechanical hyperalgesia is performed using an analgesymeter for paw pressure pain threshold. Additional direct measures of OA pain include the hind limb withdrawal test, vocalization evoked by knee compression on the affected knee, the struggle reaction to knee extension, and ambulation and rearing spontaneous movements.^{194,202–203} Weight-bearing and gait analyses may have important translational relevance for assessing OA pain because these tests are also used to assess clinical OA pain.²⁰³ However, obtaining clear pain responses from weight bearing or gait is challenging when using the unilateral DMM mouse model because the nature of OA pain is a dull pain unlike that of, for example, sharp inflammatory pain.

In large animals, pain behavior testing is more challenging and there is no consensus for the best method of evaluating pain.²⁰² However, dogs, the most commonly used large animal, have been suggested to provide the best predictive modeling for OA pain translated into the clinical setting.²⁰⁵ Methods used for assessing pain in large animals are restricted to assessing degree of lameness, gait analysis, and subjective rating scales, which assess descriptors of pain similar to those of humans.

Overall, there is a wide range of pain-behavior tests for small and large animal models. Although no animal model or pain behavior test perfectly translates to OA-associated pain in patients, these tests yield a valuable understanding of the mechanisms of OA pain and allow assessment of treatments for relief from OA-associated pain. Rodents will continue to be widely used for basic OA pain research, but large animals continue to be important because of their greater potential for modeling clinical OA pain.

FUTURE PERSPECTIVE

Although significant progress has been made in OA research in recent years, very little is yet known about the molecular mechanisms of OA initiation and progression. OA is a heterogeneous disease caused by multiple factors. One important potential factor for OA development is Runx2, which is upregulated in several OA mouse models and in cartilage samples derived from patients with OA disease.^{7–8,11,13,91} Key questions that need to be addressed are: (1) Is Runx2 a central molecule mediating OA development in joint tissue?; and (2) Could manipulation of Runx2 expression be used to treat OA disease? OA is a disease affecting the entire joint, including articular cartilage, subchondral bone, synovial tissues and menisci. In which of these joint tissues OA damage first occurs during disease initiation is currently unknown; this is important because it is directly related to OA treatment. In addition, the interplaying mechanisms among different OA

symptoms, such as articular cartilage degradation, osteophyte formation, subchondral sclerosis and synovial hyperplasia, await clarification. The understanding of the molecular mechanisms underlying these issues will accelerate the development of novel therapeutic strategies for OA.

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Competing interests

The authors declare no conflict of interest.

References

- 1 Loeser RF, Goldring SR, Scanzello CR *et al*. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum* 2012; **64**: 1697–1707.
- 2 Felson DT. Clinical practice. Osteoarthritis of the knee. *N Engl J Med* 2006; **354**: 841–848.
- 3 Goldring MB, Goldring SR. Osteoarthritis. *J Cell Physiol* 2007; **213**: 626–634.
- 4 Krasnokutsky S, Samuels J, Abramson SB. Osteoarthritis in 2007. *Bull NYU Hosp Jt Dis* 2007; **65**: 222–228.
- 5 Loeser RF. Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. *Osteoarthritis Cartilage* 2009; **17**: 971–979.
- 6 Yang X, Chen L, Xu X *et al*. TGF- β /Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J Cell Biol* 2001; **153**: 35–46.
- 7 Zhu M, Tang D, Wu Q *et al*. Activation of β -catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult β -catenin conditional activation mice. *J Bone Miner Res* 2009; **24**: 12–21.
- 8 Lin AC, Seeto BL, Bartoszko JM *et al*. Modulating hedgehog signaling can attenuate the severity of osteoarthritis. *Nat Med* 2009; **15**: 1421–1425.
- 9 Saito T, Fukai A, Mabuchi A *et al*. Transcriptional regulation of endochondral ossification by HIF-2 α during skeletal growth and osteoarthritis development. *Nat Med* 2010; **16**: 678–686.
- 10 Yang S, Kim J, Ryu JH *et al*. Hypoxia-inducible factor-2 α is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med* 2010; **16**: 687–693.
- 11 Kamekura S, Kawasaki Y, Hoshi K *et al*. Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. *Arthritis Rheum* 2006; **54**: 2462–2470.
- 12 Chen CG, Thuillier D, Chin EN *et al*. Chondrocyte-intrinsic Smad3 represses Runx2-inducible matrix metalloproteinase 13 expression to maintain articular cartilage and prevent osteoarthritis. *Arthritis Rheum* 2012; **64**: 3278–3289.
- 13 Hirata M, Kugimiya F, Fukai A *et al*. C/EBP β and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2 α as the inducer in chondrocytes. *Hum Mol Genet* 2012; **21**: 1111–1123.
- 14 Pei Y, Harvey A, Yu XP *et al*. Differential regulation of cytokine-induced MMP1 and MMP13 expression by p38 kinase inhibitors in human chondrosarcoma cells: potential role of Runx2 in mediating p38 effects. *Osteoarthritis Cartilage* 2006; **14**: 749–758.

- 15 Thirunavukkarasu K, Pei Y, Wei T. Characterization of the human ADAMTS-5 (aggrecanase-2) gene promoter. *Mol Biol Rep* 2007; **34**: 225–231.
- 16 Tetsunaga T, Nishida K, Furumatsu T *et al*. Regulation of mechanical stress-induced MMP13 and ADAMTS5 expression by Runx2 transcriptional factor in SW1353 chondrocyte-like cells. *Osteoarthritis Cartilage* 2011; **19**: 222–232.
- 17 Wang M, Tang D, Shu B *et al*. Conditional activation of β -catenin signaling in mice leads to severe defects in intervertebral disc tissue. *Arthritis Rheum* 2012; **64**: 2611–2623.
- 18 Helmick CG, Felson DT, Lawrence RC *et al*. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum* 2008; **58**: 15–25.
- 19 Lawrence RC, Felson DT, Helmick CG *et al*. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum* 2008; **58**: 26–35.
- 20 Felson DT. Osteoarthritis of the knee. *N Engl J Med* 2006; **354**: 841–848.
- 21 Felson DT, Naimark A, Anderson J *et al*. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis Rheum* 1987; **30**: 914–918.
- 22 Jordan JM, Helmick CG, Renner JB *et al*. Prevalence of knee symptoms and radiographic and symptomatic knee osteoarthritis in African Americans and Caucasians: the Johnston County Osteoarthritis Project. *J Rheumatol* 2007; **34**: 172–180.
- 23 Dillon CF, Rasch EK, Gu Q *et al*. Prevalence of knee osteoarthritis in the United States: arthritis data from the Third National Health and Nutrition Examination Survey 1991–94. *J Rheumatol* 2006; **33**: 2271–2279.
- 24 van Saase JL, van Romunde LK, Cats A *et al*. Epidemiology of osteoarthritis: Zoetermeer survey. Comparison of radiological osteoarthritis in a Dutch population with that in 10 other populations. *Ann Rheum Dis* 1989; **48**: 271–280.
- 25 Andrianakos AA, Kontelis LK, Karamitsos DG *et al*. Prevalence of symptomatic knee, hand, and hip osteoarthritis in Greece. The ESORDIG study. *J Rheumatol* 2006; **33**: 2507–2513.
- 26 Loeser RF. Aging and osteoarthritis. *Curr Opin Rheumatol* 2011; **23**: 492–496.
- 27 Kim J, Xu M, Xo R *et al*. Mitochondrial DNA damage is involved in apoptosis caused by pro-inflammatory cytokines in human OA chondrocytes. *Osteoarthritis Cartilage* 2010; **18**: 424–432.
- 28 Goodwin W, McCabe D, Sauter E *et al*. Rotenone prevents impact-induced chondrocyte death. *J Orthop Res* 2010; **28**: 1057–1063.
- 29 Naik E, Dixit VM. Mitochondrial reactive oxygen species drive pro-inflammatory cytokine production. *J Exp Med* 2011; **208**: 417–420.
- 30 Liu JT, Guo X, Ma WJ *et al*. Mitochondrial function is altered in articular chondrocytes of an endemic osteoarthritis, Kashin-Beck disease. *Osteoarthritis Cartilage* 2010; **18**: 1218–1226.
- 31 Blaney Davidson EN, Remst DF, Vitters EL *et al*. Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. *J Immunol* 2009; **182**: 7937–7945.
- 32 van der Kraan PM, Blaney Davidson EN, van den Berg WB. A role for age-related changes in TGF- β signaling in aberrant chondrocyte differentiation and osteoarthritis. *Arthritis Res Ther* 2010; **12**: 201–209.
- 33 Richardson B. Impact of aging on DNA methylation. *Ageing Res Rev* 2003; **2**: 245–261.
- 34 Christensen BC, Houseman EA, Marsit CJ *et al*. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet* 2009; **5**: e1000602.
- 35 Fernandez-Tajes J, Soto-Hermida A, Vazquez-Mosquera ME *et al*. Genome-wide DNA methylation analysis of articular chondrocytes reveals a cluster of osteoarthritic patients. *Ann Rheum Dis* 2014; **73**: 668–677.
- 36 Jeffries MA, Donica M, Baker LW *et al*. Genome-wide DNA methylation study identifies significant epigenomic changes in osteoarthritic cartilage. *Arthritis Rheumatol* 2014; **66**: 2804–2815.
- 37 den Hollander W, Ramos YF, Bos SD *et al*. Knee and hip articular cartilage have distinct epigenomic landscapes: implications for future cartilage regeneration approaches. *Ann Rheum Dis* 2014; **73**: 2208–2212.
- 38 Haseeb A, Makki MS, Haqqi TM. Modulation of ten-eleven translocation 1 (TET1), Isocitrate Dehydrogenase (IDH) expression, alpha-Ketoglutarate (alpha-KG), and DNA hydroxymethylation levels by interleukin-1beta in primary human chondrocytes. *J Biol Chem* 2014; **289**: 6877–6885.
- 39 Taylor SE, Smeriglio P, Dhulipala L *et al*. A global increase in 5-hydroxymethylcytosine levels marks osteoarthritic chondrocytes. *Arthritis Rheumatol* 2014; **66**: 90–100.
- 40 Taylor SE, Li YH, Wong WH *et al*. Genome-wide mapping of DNA hydroxymethylation in osteoarthritic chondrocytes. *Arthritis Rheumatol* 2015; **67**: 2129–2140.
- 41 Felson DT, Anderson JJ, Naimark A *et al*. Obesity and knee osteoarthritis. The Framingham Study. *Ann Intern Med* 1988; **109**: 18–24.
- 42 Anandacoomarasamy A, Caterson I, Sambrook P *et al*. The impact of obesity on the musculoskeletal system. *Int J Obes (Lond)* 2008; **32**: 211–222.
- 43 Conde J, Scotece M, Gomez R *et al*. Adipokines and osteoarthritis: novel molecules involved in the pathogenesis and progression of disease. *Arthritis* 2011; **2011**: 203901.
- 44 Das UN. Is obesity an inflammatory condition? *Nutrition* 2001; **17**: 953–966.
- 45 Fain JN. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. *Mediators Inflamm* 2010; **2010**: 513948.
- 46 Bunout D, Munoz C, Lopez M *et al*. Interleukin 1 and tumor necrosis factor in obese alcoholics compared with normal-weight patients. *Am J Clin Nutr* 1996; **63**: 373–376.
- 47 Visser M. Higher levels of inflammation in obese children. *Nutrition* 2001; **17**: 480–481.
- 48 Aygun AD, Gungor S, Ustundag B *et al*. Proinflammatory cytokines and leptin are increased in serum of prepubertal obese children. *Mediators Inflamm* 2005; **2005**: 180–183.
- 49 Pou KM, Massaro JM, Hoffmann U *et al*. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. *Circulation* 2007; **116**: 1234–1241.
- 50 Straczkowski M, Dzieńis-Straczkowska S, Stepień A *et al*. Plasma interleukin-8 concentrations are increased in obese subjects and related to fat mass and tumor necrosis factor-alpha system. *J Clin Endocrinol Metab* 2002; **87**: 4602–4606.
- 51 Zhou Q, Leeman SE, Amar S. Signaling mechanisms in the restoration of impaired immune function due to diet-induced obesity. *Proc Natl Acad Sci U S A* 2011; **108**: 2867–2872.
- 52 Neels JG, Badeanlou L, Hester KD *et al*. Keratinocyte-derived chemokine in obesity: expression, regulation, and role in adipose macrophage infiltration and glucose homeostasis. *J Biol Chem* 2009; **284**: 20692–20698.
- 53 Brown ML, Yukata K, Farnsworth CW *et al*. Delayed fracture healing and increased callus adiposity in a C57BL/6 J murine model of obesity-associated type 2 diabetes mellitus. *PLoS One* 2014; **9**: e99656.

- 54 Louer CR, Furman BD, Huebner JL *et al*. Diet-induced obesity significantly increases the severity of posttraumatic arthritis in mice. *Arthritis Rheum* 2012; **64**: 3220–3230.
- 55 Stehouwer CD, Gall MA, Twisk JW *et al*. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes* 2002; **51**: 1157–1165.
- 56 Duncan BB, Schmidt MI, Pankow JS *et al*. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003; **52**: 1799–1805.
- 57 Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; **115**: 1111–1119.
- 58 Kapoor M, Martel-Pelletier J, Lajeunesse D *et al*. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011; **7**: 33–42.
- 59 Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. *Biorheology* 2002; **39**: 237–246.
- 60 Martel-Pelletier J, Alaeddine N, Pelletier JP. Cytokines and their role in the pathophysiology of osteoarthritis. *Front Biosci* 1999; **4**: D694–D703.
- 61 Roos EM. Joint injury causes knee osteoarthritis in young adults. *Curr Opin Rheumatol* 2005; **17**: 195–200.
- 62 Radin EL. Who gets osteoarthritis and why? *J Rheumatol Suppl*, 2004; **70**: 10–15.
- 63 Andriacchi TP, Mundermann A, Smith RL *et al*. A framework for the *in vivo* pathomechanics of osteoarthritis at the knee. *Ann Biomed Eng* 2004; **32**: 447–457.
- 64 Miyazaki T, Wada M, Kawahara H *et al*. Dynamic load at baseline can predict radiographic disease progression in medial compartment knee osteoarthritis. *Ann Rheum Dis* 2002; **61**: 617–622.
- 65 Fridén T, Sommerlath K, Egund N *et al*. Instability after anterior cruciate ligament rupture. Measurements of sagittal laxity compared in 11 cases. *Acta Orthop Scand* 1992; **63**: 593–598.
- 66 Sernert N, Kartus JT Jr, Ejerhed L *et al*. Right and left knee laxity measurements: a prospective study of patients with anterior cruciate ligament injuries and normal control subjects. *Arthroscopy* 2004; **20**: 564–571.
- 67 Lieberthal J, Sambamurthy N, Scanzello CR. Inflammation in joint injury and post-traumatic osteoarthritis. *Osteoarthritis Cartilage* 2015; **23**: 1825–1834.
- 68 Malfait AM. Osteoarthritis year in review 2015: biology. *Osteoarthritis Cartilage* 2016; **24**: 21–26.
- 69 van Lent PL, Blom AB, Schelbergen RF *et al*. Active involvement of alarmins S100A8 and S100A9 in the regulation of synovial activation and joint destruction during mouse and human osteoarthritis. *Arthritis Rheum* 2012; **64**: 1466–1476.
- 70 Schelbergen RF, van Dalen S, ter Huurne M *et al*. Treatment efficacy of adipose-derived stem cells in experimental osteoarthritis is driven by high synovial activation and reflected by S100A8/A9 serum levels. *Osteoarthritis Cartilage* 2014; **22**: 1158–1166.
- 71 Nasi S, Ea HK, Chobaz V *et al*. Dispensable role of myeloid differentiation primary response gene 88 (MyD88) and MyD88-dependent toll-like receptors (TLRs) in a murine model of osteoarthritis. *Joint Bone Spine* 2014; **81**: 320–324.
- 72 Liu-Bryan R, Terkeltaub R. The growing array of innate inflammatory ignition switches in osteoarthritis. *Arthritis Rheum* 2012; **64**: 2055–2058.
- 73 Schelbergen RF, Blom AB, van den Bosch MH *et al*. Alarmins S100A8 and S100A9 elicit a catabolic effect in human osteoarthritic chondrocytes that is dependent on Toll-like receptor 4. *Arthritis Rheum* 2012; **64**: 1477–1487.
- 74 Zreiqat H, Belluocchio D, Smith MM *et al*. S100A8 and S100A9 in experimental osteoarthritis. *Arthritis Res Ther* 2010; **12**: R16.
- 75 Cecil DL, Appleton CT, Polewski MD *et al*. The pattern recognition receptor CD36 is a chondrocyte hypertrophy marker associated with suppression of catabolic responses and promotion of repair responses to inflammatory stimuli. *J Immunol* 2009; **182**: 5024–5031.
- 76 Jin C, Frayssinet P, Pelker R *et al*. NLRP3 inflammasome plays a critical role in the pathogenesis of hydroxyapatite-associated arthropathy. *Proc Natl Acad Sci U S A* 2011; **108**: 14867–14872.
- 77 Wang Q, Rozelle AL, Lepus CM *et al*. Identification of a central role for complement in osteoarthritis. *Nat Med* 2011; **17**: 1674–1679.
- 78 Lepus CM, Song JJ, Wang Q *et al*. Brief report: carboxypeptidase B serves as a protective mediator in osteoarthritis. *Arthritis Rheumatol* 2014; **66**: 101–106.
- 79 Pap T, Bertrand J. Syndecans in cartilage breakdown and synovial inflammation. *Nat Rev Rheumatol* 2013; **9**: 43–55.
- 80 Kim JH, Jeon J, Shin M *et al*. Regulation of the catabolic cascade in osteoarthritis by the zinc-ZIP8-MTF1 axis. *Cell* 2014; **156**: 730–743.
- 81 Kroemer G. Autophagy: a druggable process that is deregulated in aging and human disease. *J Clin Invest* 2015; **125**: 1–4.
- 82 Lopez-Otin C, Blasco MA, Partridge L *et al*. The hallmarks of aging. *Cell* 2013; **153**: 1194–1217.
- 83 Carames B, Olmer M, Kiosses WB *et al*. The relationship of autophagy defects to cartilage damage during joint aging in a mouse model. *Arthritis Rheumatol* 2015; **67**: 1568–1576.
- 84 Vasheghani F, Zhang Y, Li YH *et al*. PPAR γ deficiency results in severe, accelerated osteoarthritis associated with aberrant mTOR signalling in the articular cartilage. *Ann Rheum Dis* 2015; **74**: 569–578.
- 85 Takayama K, Kawakami Y, Kobayashi M *et al*. Local intra-articular injection of rapamycin delays articular cartilage degeneration in a murine model of osteoarthritis. *Arthritis Res Ther* 2014; **16**: 482–491.
- 86 Li ZC, Xiao J, Peng JL *et al*. Functional annotation of rheumatoid arthritis and osteoarthritis associated genes by integrative genome-wide gene expression profiling analysis. *PLoS One* 2014; **9**: e85784.
- 87 Spector TD, Cicuttini F, Baker J *et al*. Genetic influences on osteoarthritis in women: a twin study. *BMJ* 1996; **312**: 940–943.
- 88 Felson DT, Couropmitree NN, Chaisson CE *et al*. Evidence for a Mendelian gene in a segregation analysis of generalized radiographic osteoarthritis: the Framingham Study. *Arthritis Rheum* 1998; **41**: 1064–1071.
- 89 Loughlin J, Mustafa Z, Smith A *et al*. Linkage analysis of chromosome 2q in osteoarthritis. *Rheumatology* 2000; **39**: 377–381.
- 90 Serra R, Johnson M, Filvaroff EH *et al*. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol* 1997; **139**: 541–552.
- 91 Shen J, Li J, Wang B *et al*. Deletion of the transforming growth factor beta receptor type II gene in articular chondrocytes leads to a progressive osteoarthritis-like phenotype in mice. *Arthritis Rheum* 2013; **65**: 3107–3119.
- 92 Wang M, Tang D, Shu B *et al*. Conditional activation of beta-catenin signaling in mice leads to severe defects in intervertebral disc tissue. *Arthritis Rheum* 2012; **64**: 2611–2623.
- 93 Miranda AJ, Liu Z, Moore T *et al*. RBP-Jkappa-dependent Notch signaling is required for murine articular cartilage and joint maintenance. *Arthritis Rheum* 2013; **65**: 2623–2633.
- 94 Lories RJ, Corr M, Lane NE. To Wnt or not to Wnt: the bone and joint health dilemma. *Nat Rev Rheumatol* 2013; **9**: 328–339.

- 95 Sassi N, Laadhar L, Allouche M *et al*. WNT signaling and chondrocytes: from cell fate determination to osteoarthritis physiopathology. *J Recept Signal Transduct Res* 2014; **34**: 73–80.
- 96 Hirata M, Kugimiya F, Fukai A *et al*. C/EBPbeta and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2alpha as the inducer in chondrocytes. *Hum Mol Genet* 2012; **21**: 1111–1123.
- 97 Little CB, Barai A, Burkhardt D *et al*. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. *Arthritis Rheum* 2009; **60**: 3723–3733.
- 98 Glasson SS, Askew R, Sheppard B *et al*. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* 2005; **434**: 644–648.
- 99 Barnholtz-Sloan JS, Severson RK, Stanton B *et al*. Pediatric brain tumors in non-Hispanics, Hispanics, African Americans and Asians: differences in survival after diagnosis. *Cancer Causes Control* 2005; **16**: 587–592.
- 100 Loughlin J, Dowling B, Chapman K *et al*. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. *Proc Natl Acad Sci U S A* 2004; **101**: 9757–9762.
- 101 Bijsterbosch J, Kloppenburg M, Reijnen M *et al*. Association study of candidate genes for the progression of hand osteoarthritis. *Osteoarthritis Cartilage* 2013; **21**: 565–569.
- 102 Valdes AM, Spector TD, Tamm A *et al*. Genetic variation in the SMAD3 gene is associated with hip and knee osteoarthritis. *Arthritis Rheum* 2010; **62**: 2347–2352.
- 103 Rodriguez RR, Seegmiller RE, Stark MR *et al*. A type XI collagen mutation leads to increased degradation of type II collagen in articular cartilage. *Osteoarthritis Cartilage* 2004; **12**: 314–320.
- 104 Jeong C, Lee JY, Kim J *et al*. Novel COL9A3 mutation in a family diagnosed with multiple epiphyseal dysplasia: a case report. *BMC Musculoskelet Disord* 2014; **15**: 371.
- 105 Carlson KM, Yamaga KM, Reinker KA *et al*. Precocious osteoarthritis in a family with recurrent COL2A1 mutation. *J Rheumatol* 2006; **33**: 1133–1136.
- 106 Zhang R, Yao J, Xu P *et al*. A comprehensive meta-analysis of association between genetic variants of GDF5 and osteoarthritis of the knee, hip and hand. *Inflamm Res* 2015; **64**: 405–414.
- 107 arcOGEN Consortium and arcOGEN Collaborators. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. *Lancet* 2012; **380**: 815–823.
- 108 Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis Cartilage* 2007; **15**: 1061–1069.
- 109 Glasson SS, Askew R, Sheppard B *et al*. Characterization of and osteoarthritis susceptibility in ADAMTS-4-knockout mice. *Arthritis Rheum* 2004; **50**: 2547–2558.
- 110 Welch ID, Cowan MF, Beier F *et al*. The retinoic acid binding protein CRABP2 is increased in murine models of degenerative joint disease. *Arthritis Res Ther* 2009; **11**: R14.
- 111 Ma HL, Blanchet TJ, Peluso D *et al*. Osteoarthritis severity is sex dependent in a surgical mouse model. *Osteoarthritis Cartilage* 2007; **15**: 695–700.
- 112 Wilhelmi G, Faust R. Suitability of the C57 black mouse as an experimental animal for the study of skeletal changes due to ageing, with special reference to osteo-arthrosis and its response to tribenoside. *Pharmacology* 1976; **14**: 289–296.
- 113 Walton M. Studies of degenerative joint disease in the mouse knee joint; scanning electron microscopy. *J Pathol* 1977; **123**: 211–217.
- 114 Poulet B, Westerhof TA, Hamilton RW *et al*. Spontaneous osteoarthritis in Str/ort mice is unlikely due to greater vulnerability to mechanical trauma. *Osteoarthritis Cartilage* 2013; **21**: 756–763.
- 115 Poulet B, Ulici V, Stone TC *et al*. Time-series transcriptional profiling yields new perspectives on susceptibility to murine osteoarthritis. *Arthritis Rheum* 2012; **64**: 3256–3266.
- 116 Sokoloff J, Crittenden LB, Yamamoto RS *et al*. The genetics of degenerative joint disease in mice. *Arthritis Rheum* 1962; **5**: 531–546.
- 117 Kamekura S, Hoshi K, Shimoaka T *et al*. Osteoarthritis development in novel experimental mouse models induced by knee joint instability. *Osteoarthritis Cartilage* 2005; **13**: 632–641.
- 118 Jimenez PA, Glasson SS, Trubetsky OV *et al*. Spontaneous osteoarthritis in Dunkin Hartley guinea pigs: histologic, radiologic, and biochemical changes. *Lab Anim Sci* 1997; **47**: 598–601.
- 119 Thijssen E, van Caam A, van der Kraan PM. Obesity and osteoarthritis, more than just wear and tear: pivotal roles for inflamed adipose tissue and dyslipidaemia in obesity-induced osteoarthritis. *Rheumatology* 2015; **54**: 588–600.
- 120 Griffin TM, Huebner JL, Kraus VB *et al*. Induction of osteoarthritis and metabolic inflammation by a very high-fat diet in mice: effects of short-term exercise. *Arthritis Rheum* 2012; **64**: 443–453.
- 121 Säämänen AM, Hyttinen M, Vuorio E. Analysis of arthritic lesions in the Del1 mouse: a model for osteoarthritis. *Methods Mol Med* 2007; **136**: 283–302.
- 122 Chen M, Lichtler AC, Sheu T *et al*. Generation of a transgenic mouse model with chondrocyte-specific and tamoxifen-inducible expression of Cre recombinase. *Genesis* 2007; **45**: 44–50.
- 123 Henry SP, Jang CW, Deng JM *et al*. Generation of aggrecan-CreERT2 knockin mice for inducible Cre activity in adult cartilage. *Genesis* 2009; **47**: 805–814.
- 124 Kozhemyakina E, Zhang M, Ionescu A *et al*. Identification of a Prg4-expressing articular cartilage progenitor cell population in mice. *Arthritis Rheumatol* 2015; **67**: 1261–1273.
- 125 Saamanen AK, Salminen HJ, Dean PB *et al*. Osteoarthritis-like lesions in transgenic mice harboring a small deletion mutation in type II collagen gene. *Osteoarthritis Cartilage* 2000; **8**: 248–257.
- 126 Hu K, Xu L, Cao L *et al*. Pathogenesis of osteoarthritis-like changes in the joints of mice deficient in type IX collagen. *Arthritis Rheum* 2006; **54**: 2891–2900.
- 127 Wu Q, Kim KO, Sampson ER *et al*. Induction of an osteoarthritis-like phenotype and degradation of phosphorylated Smad3 by Smurf2 in transgenic mice. *Arthritis Rheum* 2008; **58**: 3132–3144.
- 128 Lories RJ, Peeters J, Bakker A *et al*. Articular cartilage and biomechanical properties of the long bones in Frzb-knockout mice. *Arthritis Rheum* 2007; **56**: 4095–4103.
- 129 Weng T, Yi L, Huang J *et al*. Genetic inhibition of fibroblast growth factor receptor 1 in knee cartilage attenuates the degeneration of articular cartilage in adult mice. *Arthritis Rheum* 2012; **64**: 3982–3992.
- 130 Wang M, Sampson ER, Jin H *et al*. MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis Res Ther* 2013; **15**: R5.
- 131 Valdes AM, Spector TD, Tamm A *et al*. Genetic variation in the Smad3 gene is associated with hip and knee osteoarthritis. *Arthritis Rheum* 2010; **62**: 2347–2352.
- 132 van de Laar IM, Oldenburg RA, Pals G *et al*. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. *Nat Genet* 2011; **43**: 121–126.
- 133 van de Laar IM, van der Linde D, Oei EH *et al*. Phenotypic spectrum of the Smad3-related aneurysms-osteoarthritis syndrome. *J Med Genet* 2012; **49**: 47–57.

- 134 Zhen G, Wen C, Jia X *et al*. Inhibition of TGF- β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. *Nat Med* 2013; **19**: 704–712.
- 135 Wang TY, Chen D. Differential roles of TGF- β signaling in joint tissues during osteoarthritis development. *Ann Rheum Dis* 2016; **75**: e72.
- 136 Manning WK, Bonner WM Jr. Isolation and culture of chondrocytes from human adult articular cartilage. *Arthritis Rheum* 1967; **10**: 235–239.
- 137 Oseni AO, Butler PE, Seifalian AM. Optimization of chondrocyte isolation and characterization for large-scale cartilage tissue engineering. *J Surg Res* 2013; **181**: 41–48.
- 138 Guo JF, Jourdan GW, MacCallum DK. Culture and growth characteristics of chondrocytes encapsulated in alginate beads. *Connect Tissue Res* 1989; **19**: 277–297.
- 139 Bonaventure J, Kadhom N, Cohen-Solal L *et al*. Reexpression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. *Exp Cell Res* 1994; **212**: 97–104.
- 140 Lafeber FP, Vander Kraan PM, Van Roy JL *et al*. Articular cartilage explant culture; an appropriate *in vitro* system to compare osteoarthritic and normal human cartilage. *Connect Tissue Res* 1993; **29**: 287–299.
- 141 Dhillon RS, Zhang L, Schwarz EM *et al*. The murine femoral bone graft model and a semiautomated histomorphometric analysis tool. *Methods Mol Biol* 2014; **1130**: 45–59.
- 142 Glasson SS, Chambers MG, Van Den Berg WB *et al*. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage* 2010; **18**: S17–S23.
- 143 Waldstein W, Perino G, Gilbert SL *et al*. OARSI osteoarthritis cartilage histopathology assessment system: A biomechanical evaluation in the human knee. *J Orthop Res* 2016; **34**: 135–140.
- 144 Han L, Grodzinsky AJ, Ortiz C. Nanomechanics of the cartilage extracellular matrix. *Annu Rev Mater Res* 2011; **41**: 133–168.
- 145 Lin DC, Horkay F. Nanomechanics of polymer gels and biological tissues: a critical review of analytical approaches in the Hertzian regime and beyond. *Soft Matter* 2008; **4**: 669–682.
- 146 McLeod MA, Wilusz RE, Guilak F. Depth-dependent anisotropy of the micromechanical properties of the extracellular and pericellular matrices of articular cartilage evaluated via atomic force microscopy. *J Biomech* 2013; **46**: 586–592.
- 147 Kwok J, Grogan S, Meckes B *et al*. Atomic force microscopy reveals age-dependent changes in nanomechanical properties of the extracellular matrix of native human menisci: implications for joint degeneration and osteoarthritis. *Nanomedicine* 2014; **10**: 1777–1785.
- 148 Stolz M, Gottardi R, Raiteri R *et al*. Early detection of aging cartilage and osteoarthritis in mice and patient samples using atomic force microscopy. *Nat Nanotechnol* 2009; **4**: 186–192.
- 149 Wilusz RE, Zauscher S, Guilak F. Micromechanical mapping of early osteoarthritic changes in the pericellular matrix of human articular cartilage. *Osteoarthritis Cartilage* 2013; **21**: 1895–1903.
- 150 Desrochers J, Amrein MA, Matyas JR. Structural and functional changes of the articular surface in a post-traumatic model of early osteoarthritis measured by atomic force microscopy. *J Biomech* 2010; **43**: 3091–3098.
- 151 Desrochers J, Amrein MW, Matyas JR. Viscoelasticity of the articular cartilage surface in early osteoarthritis. *Osteoarthritis Cartilage* 2012; **20**: 413–421.
- 152 Darling EM, Topel M, Zauscher S *et al*. Viscoelastic properties of human mesenchymally-derived stem cells and primary osteoblasts, chondrocytes, and adipocytes. *J Biomech* 2008; **41**: 454–464.
- 153 Lee B, Han L, Frank EH *et al*. Dynamic nanomechanics of individual bone marrow stromal cells and cell-matrix composites during chondrogenic differentiation. *J Biomech* 2015; **48**: 171–175.
- 154 Diekman BO, Christoforou N, Willard VP *et al*. Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. *Proc Natl Acad Sci U S A* 2012; **109**: 19172–19177.
- 155 Ng L, Hung H-H, Sprunt A *et al*. Nanomechanical properties of individual chondrocytes and their developing growth factor-stimulated pericellular matrix. *J Biomech* 2007; **40**: 1011–1023.
- 156 Peñuela L, Wolf F, Raiteri R *et al*. Atomic force microscopy to investigate spatial patterns of response to interleukin-1beta in engineered cartilage tissue elasticity. *J Biomech* 2014; **47**: 2157–2164.
- 157 Ameye LG, Young MF. Animal models of osteoarthritis: lessons learned while seeking the ‘Holy Grail’. *Curr Opin Rheumatol* 2006; **18**: 537–547.
- 158 Coles JM, Zhang L, Blum JJ *et al*. Loss of cartilage structure, stiffness, and frictional properties in mice lacking PRG4. *Arthritis Rheum* 2010; **62**: 1666–1674.
- 159 Batista MA, Nia HT, Önnnerfjord P *et al*. Nanomechanical phenotype of chondroadherin-null murine articular cartilage. *Matrix Biol* 2014; **38**: 84–90.
- 160 Li Q, Doyran B, Gamer LW *et al*. Biomechanical properties of murine meniscus surface via AFM-based nanoindentation. *J Biomech* 2015; **48**: 1364–1370.
- 161 Wilusz RE, DeFrate LE, Guilak F. Immunofluorescence-guided atomic force microscopy to measure the micromechanical properties of the pericellular matrix of porcine articular cartilage. *J R Soc Interface* 2012; **9**: 2997–3007.
- 162 Wilusz RE, DeFrate LE, Guilak F. A biomechanical role for perlecan in the pericellular matrix of articular cartilage. *Matrix Biol* 2012; **31**: 320–327.
- 163 Wilusz RE, Guilak F. High resistance of the mechanical properties of the chondrocyte pericellular matrix to proteoglycan digestion by chondroitinase, aggrecanase, or hyaluronidase. *J Mech Behav Biomed Mater* 2014; **38**: 183–197.
- 164 Zelenski NA, Leddy HA, Sanchez-Adams J *et al*. Type VI collagen regulates pericellular matrix properties, chondrocyte swelling, and mechanotransduction in mouse articular cartilage. *Arthritis Rheumatol* 2015; **67**: 1286–1294.
- 165 Sanchez-Adams J, Wilusz RE, Guilak F. Atomic force microscopy reveals regional variations in the micromechanical properties of the pericellular and extracellular matrices of the meniscus. *J Orthop Res* 2013; **31**: 1218–1225.
- 166 Nia HT, Bozchalooi IS, Li Y *et al*. High-bandwidth AFM-based rheology reveals that cartilage is most sensitive to high loading rates at early stages of impairment. *Biophys J* 2013; **104**: 1529–1537.
- 167 Han L, Frank EH, Greene JJ *et al*. Time-dependent nanomechanics of cartilage. *Biophys J* 2011; **100**: 1846–1854.
- 168 Nia HT, Han L, Li Y *et al*. Poroelasticity of cartilage at the nanoscale. *Biophys J* 2011; **101**: 2304–2313.
- 169 Nia HT, Han L, Bozchalooi IS *et al*. Aggrecan nanoscale solid-fluid interactions are a primary determinant of cartilage dynamic mechanical properties. *ACS Nano* 2015; **9**: 2614–2625.
- 170 Nia HT, Gauci SJ, Azadi M *et al*. High-bandwidth AFM-based rheology is a sensitive indicator of early cartilage aggrecan degradation relevant to mouse models of osteoarthritis. *J Biomech* 2015; **48**: 162–165.
- 171 Im HJ, Kim JS, Li X *et al*. Alteration of sensory neurons and spinal response to an experimental osteoarthritis pain model. *Arthritis Rheum* 2010; **62**: 2995–3005.

- 172 Lee AS, Ellman MB, Yan D *et al*. A current review of molecular mechanisms regarding osteoarthritis and pain. *Gene* 2013; **527**: 440–447.
- 173 Deshmane SL, Kremlev S, Amini S *et al*. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* 2009; **29**: 313–326.
- 174 Harigai M, Hara M, Yoshimura T *et al*. Monocyte chemoattractant protein-1 (MCP-1) in inflammatory joint diseases and its involvement in the cytokine network of rheumatoid synovium. *Clin Immunol Immunopathol* 1993; **69**: 83–91.
- 175 Thompson WL, Karpus WJ, Van Eldik LJ. MCP-1-deficient mice show reduced neuroinflammatory responses and increased peripheral inflammatory responses to peripheral endotoxin insult. *J Neuroinflammation* 2008; **5**: 35–47.
- 176 Conductier G, Blondeau N, Guyon A *et al*. The role of monocyte chemoattractant protein MCP1/CCL2 in neuroinflammatory diseases. *J Neuroimmunol* 2010; **224**: 93–100.
- 177 Thacker MA, Clark AK, Bishop T *et al*. CCL2 is a key mediator of microglia activation in neuropathic pain states. *Eur J Pain* 2009; **13**: 263–272.
- 178 Van Steenwinckel J, Reaus-Le Goazigo A, Pommier B *et al*. CCL2 released from neuronal synaptic vesicles in the spinal cord is a major mediator of local inflammation and pain after peripheral nerve injury. *J Neurosci* 2011; **31**: 5865–5875.
- 179 Miller RE, Tran PB, Das R *et al*. CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis. *Proc Natl Acad Sci U S A* 2012; **109**: 20602–20607.
- 180 Horuk R. Chemokine receptor antagonists: overcoming developmental hurdles. *Nat Rev Drug Discov* 2009; **8**: 23–33.
- 181 Lane NE, Schnitzer TJ, Birbara CA *et al*. Tanezumab for the treatment of pain from osteoarthritis of the knee. *N Engl J Med* 2010; **363**: 1521–1531.
- 182 Schnitzer TJ, Lane NE, Birbara C *et al*. Long-term open-label study of tanezumab for moderate to severe osteoarthritic knee pain. *Osteoarthritis Cartilage* 2011; **19**: 639–646.
- 183 Brown MT, Murphy FT, Radin DM *et al*. Tanezumab reduces osteoarthritic knee pain: results of a randomized, double-blind, placebo-controlled phase III trial. *J Pain* 2012; **13**: 790–798.
- 184 Nagashima H, Suzuki M, Araki S *et al*. Preliminary assessment of the safety and efficacy of tanezumab in Japanese patients with moderate to severe osteoarthritis of the knee: a randomized, double-blind, dose-escalation, placebo-controlled study. *Osteoarthritis Cartilage* 2011; **19**: 1405–1412.
- 185 Obata K, Noguchi K. MAPK activation in nociceptive neurons and pain hypersensitivity. *Life Sci* 2004; **74**: 2643–2653.
- 186 McKelvey L, Shorten GD, O'Keefe GW. Nerve growth factor-mediated regulation of pain signalling and proposed new intervention strategies in clinical pain management. *J Neurochem* 2013; **124**: 276–289.
- 187 Hefti FF, Rosenthal A, Walicke PA *et al*. Novel class of pain drugs based on antagonism of NGF. *Trends Pharmacol Sci* 2006; **27**: 85–91.
- 188 Mantyh PW, Koltzenburg M, Mendell LM *et al*. Antagonism of nerve growth factor-TrkA signaling and the relief of pain. *Anesthesiology* 2011; **115**: 189–204.
- 189 Kawamoto K, Aoki J, Tanaka A *et al*. Nerve growth factor activates mast cells through the collaborative interaction with lysophosphatidylserine expressed on the membrane surface of activated platelets. *J Immunol* 2002; **168**: 6412–6419.
- 190 Seidel MF, Wise BL, Lane NE. Nerve growth factor: an update on the science and therapy. *Osteoarthritis Cartilage* 2013; **21**: 1223–1228.
- 191 Gee AP, Boyle MD, Munger KL *et al*. Nerve growth factor: stimulation of polymorphonuclear leukocyte chemotaxis *in vitro*. *Proc Natl Acad Sci U S A* 1983; **80**: 7215–7218.
- 192 Otten U, Baumann JB, Girard J. Nerve growth factor induces plasma extravasation in rat skin. *Eur J Pharmacol* 1984; **106**: 199–201.
- 193 Walsh DA, McWilliams DF, Turley MJ *et al*. Angiogenesis and nerve growth factor at the osteochondral junction in rheumatoid arthritis and osteoarthritis. *Rheumatology* 2010; **49**: 1852–1861.
- 194 Kc R, Li X, Kroin JS *et al*. PKC δ null mutations in a mouse model of osteoarthritis alter osteoarthritic pain independently of joint pathology by augmenting NGF/TrkA-induced axonal outgrowth. *Ann Rheum Dis* 2016; **75**: 2133–2141.
- 195 Hochberg MC. Serious joint-related adverse events in randomized controlled trials of anti-nerve growth factor monoclonal antibodies. *Osteoarthritis Cartilage* 2015; **23**: S18–S21.
- 196 Malfait AM, Ritchie J, Gil AS *et al*. ADAMTS-5 deficient mice do not develop mechanical allodynia associated with osteoarthritis following medial meniscal destabilization. *Osteoarthritis Cartilage* 2010; **18**: 572–580.
- 197 Miller RE, Tran PB, Ishihara S *et al*. Therapeutic effects of an anti-ADAMTS-5 antibody on joint damage and mechanical allodynia in a murine model of osteoarthritis. *Osteoarthritis Cartilage* 2016; **24**: 299–306.
- 198 Verma P, Dalal K. ADAMTS-4 and ADAMTS-5: key enzymes in osteoarthritis. *J Cell Biochem* 2011; **112**: 3507–3514.
- 199 Chan CC, Roberts CR, Steeves JD *et al*. Aggrecan components differentially modulate nerve growth factor-responsive and neurotrophin-3-responsive dorsal root ganglion neurite growth. *J Neurosci Res* 2008; **86**: 581–592.
- 200 Mancuso CA, Ranawat CS, Esdaile JM *et al*. Indications for total hip and total knee arthroplasties. Results of orthopaedic surveys. *J Arthroplasty* 1996; **11**: 34–46.
- 201 Hunter DJ, McDougall JJ, Keefe FJ. The symptoms of osteoarthritis and the genesis of pain. *Med Clin North Am* 2009; **93**: 83–100.
- 202 Piel MJ, Kroin JS, van Wijnen AJ *et al*. Pain assessment in animal models of osteoarthritis. *Gene* 2014; **537**: 184–188.
- 203 Neugebauer V, Han JS, Adwanikar H *et al*. Techniques for assessing knee joint pain in arthritis. *Mol Pain* 2007; **3**: 8.
- 204 Malfait AM, Little CB, McDougall JJ. A commentary on modelling osteoarthritic pain in small animals. *Osteoarthritis Cartilage* 2013; **21**: 1316–1326.
- 205 Henze DA, Urban MO. Large animal models for pain therapeutic development. In: Kruger L, Light AR(ed.). *Translational Pain Research: From Mouse to Man*. London: CRC Press, 2010: 371–390.



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