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Do mouse models reflect the diversity of osteoarthritis in humans?

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While osteoarthritis (OA) is a “whole joint” disorder involving all joint tissues, progressive cartilage erosion is the major pathological indicator leading to joint replacement surgery. We have learned much about the molecular mechanisms driving cartilage destruction from studies of gene and protein expression in clinical material and in cell culture models, derived from human tissues. However, it is not possible to follow human patients longitudinally with regard to changes in gene expression in joint tissues *in situ*, since observations must be made as endpoints in operative specimens. Furthermore, biomarkers in synovial fluid, serum, and urine, as well as MRI, for early diagnosis or therapeutic outcome have yet to be fully characterized for use in cohorts, let alone in individual patients.

The use of genetically modified mice and comparisons of different mouse strains have permitted validation of many of the mechanisms associated with risk factors such as biomechanical instability, injury, inflammation, obesity, and genetic polymorphisms. Surgical OA models that reflect some aspects of post-traumatic OA in humans have been useful particularly for studying the consequences of knockout or transgenic overexpression of genes of interest in mice (1, 2). As in human genetic disorders, such as chondrodysplasias, genetic mouse models with abnormal composition and structure of articular cartilage or other joint tissues may develop spontaneous or accelerated OA due to altered biomechanics. Although *in vivo* experiments are subject to interpretation and mouse models may not recapitulate all aspects of human disease, they do allow us to study the time course of the disease in a way that is not possible in humans. Common to all of these models are certain molecular pathways, particularly those controlling expression and activation of proteolytic enzymes, which have been identified as determining initiation and progression of cartilage damage. Gene profiling studies in experimental OA models have provided additional targets for consideration (3–7).

The paper by Poulet et al. (8) in this issue reports an interesting and well-executed study that adds to our arsenal of gene regulatory mechanisms in mouse OA models with application to human disease. The data reported show appropriate comparisons of gene expression in articular cartilage that allow conclusions to be made about differences during aging between OA-susceptible (Str/ort) mice and non-susceptible (CBA) mice as controls. The Str/ort mouse is a model of spontaneous OA that develops with aging and the analyses reported here show a molecular phenotype resembling that reported in profiling studies of human OA cartilage (9, 10), in which both catabolic and anabolic gene signatures have been identified. Comparing Str/ort mice at 18 and 40 weeks of age with those at 8 weeks that had not yet developed OA and the CBA controls at the 3 ages yielded three sets of gene signatures,

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including those involving known NF- κ B pathway associated genes, but also an immune cell signature and a surprisingly robust myogenic signature.

Pathway analysis revealed that the NF- κ B pathway-related immunological and inflammatory signatures were present in Str/ort chondrocytes before the appearance of overt OA and associated with increased numbers of activated T and B lymphocytes in the spleens of 8-week-old mice (8). The prominence of the NF- κ B pathway as a common thread among the different gene signatures has been emphasized in other studies suggesting a key regulatory role for canonical NF- κ B signalling in human OA (see for review (11, 12)). Although OA occurs in Str/ort mice without overt inflammation such as synovitis or immune cell infiltration, the NF- κ B pathway may be modulated positively or negatively by mechanical stimuli (13).

The skeletal muscle-specific isoform expression of genes encoding structural, metabolic, and matrix proteins is remarkable in that it was up-regulated during cartilage maturation in both mouse strains, but was higher in the CBA strain; however, in aging CBA mice it was switched off, but retained in the Str/ort mice during OA development (8). The authors concluded that failure to switch off the muscle gene expression program was related to NF- κ B pathway activation, which acts as a hub for driving gene expression during OA development and progression. Loeser et al, (7) also found changes in muscle-specific gene expression in older but not in young mice subjected to surgically induced OA. Differences in gene profiles may be attributed to the fact that the tissues analyzed in that study included bone, joint capsule, and other tissues in addition to cartilage, although periostin, one of the prominent genes identified, also appears in the NF- κ B complex in the 8-week-old Str/ort mice in the study by Poulet et al (8). In addition, the C57BL6 strain used by Loeser et al. (7) does develop OA with aging, but OA onset and progression are accelerated in the post-traumatic OA model.

Glasson (1) screened several mouse strains in the surgical destabilization of the medial meniscus (DMM) model, used in most of the studies mentioned above, and found that 129/SvEv mice were most susceptible, whereas the least susceptible strain tested was DBA/1, which is highly susceptible to inflammatory arthritis. The C57BL/6 strain, used most frequently for generation of knockout and transgenic mice, had intermediate susceptibility, indicating its utility whether the genetic modification is expected to enhance or attenuate OA following challenge. Age-related changes in cartilage are expected to occur in all mouse strains, but do not inevitably lead to OA, as in CBA mice. Furthermore, differences in gene signatures were observed between strains, but there was no difference observed in the study by Poulet et al. (8) when early and late OA profiles were compared in Str/ort mice. What appears to distinguish strains that are OA-prone is the time of onset and the severity of OA progression and the authors hypothesized that a specific molecular program, as identified in Str/ort mice, will elucidate interactions between ageing and the OA phenotypes that generate increased risk of disease in some individuals. Since most OA-related genes, except for CILP, did not change with age in the CBA strain, the OA signature may be specific to the disease and unrelated to aging alone. These findings lead credence to the possibility of identifying gene signatures in at-risk populations prior to the onset of overt OA.

Although comparisons of different mouse strains may accentuate differences in gene expression unrelated to disease status, such analyses may reflect the complexities in different human OA populations as exemplified in GWAS studies. Another recently published study highlights how strain-related differences in mice may mirror differences in OA susceptibility in humans. In the study by Hashimoto et al. (14), two recombinant inbred strains selected for articular cartilage regenerating and ear wound healing capacity were subjected to surgical OA. The non-healer mice developed a significantly higher grade of OA

than the healer mice, which are related to the MRL/MpJ superhealer strain, as well as increased subchondral bone and trabecular bone thickening at 8-weeks post-surgery. A related study by Rai et al. (15) showed that articular cartilage regeneration is heritable and together the two studies indicate that the phenotypic differences in OA development and regenerative capacity are due to genetic differences that may provide insight into how to dissect the genetic basis of variation in susceptible individuals.

It is also important to understand strain differences because of the increasing use of pre-clinical models for testing therapies for translation to the clinic. Treatments with antibody against syndecan-4 (16), a Hedgehog signaling inhibitor (17), recombinant human PTH(1-34) (teriparatide) (18), an aggrecanase inhibitor (19), and kartogenin, a small molecule targeting the chondrogenic program (20), have been shown to reduce the severity of OA, and in some cases to promote regeneration, in surgically induced OA models in mice or rats.

Of interest in the context of clinical translation is that physical function and pain sensitivity can be analyzed in mouse OA models. Allen et al. (21) showed compensatory gait changes, increased mechanical sensitivity, and impaired physical ability in *Col9a1*^{-/-} mice, a model of genetic OA. Alternative to the invasive DMM model are injury-induced models in which repetitive loading causes cartilage damage, such as that reported previously by Poulet et al. (22). In contrast, physiological loading of joints may prevent cartilage damage (23), whereas immobilization of joints by sciatic neurectomy abrogated OA development in a DMM model and demonstrated the mechanosensitivity of proteinase expression in microarray analysis (24). Ex vivo modeling of acute cartilage injury and microarray analysis (25) revealed the prominent role of Wnt/ β -catenin signaling in the development of OA, consistent with findings from a microarray analysis of mice deficient in the *FrzB* gene encoding a Wnt inhibitor that is important in the maintenance of cartilage homeostasis (5). A previous editorial by Vincent and Saklatvala (26), however, pointed out the prominence of repair response genes in late OA in both human cartilage and in surgical OA models in contrast to the non-invasive cartilage injury models, which may exhibit more obvious bone phenotypes.

Gene array studies, however, have limitations because they indicate associations that must be proven by detailed mechanistic studies. For example, the elevated NF- κ B/p65 protein analyzed by immunohistochemistry in the Str/Ort cartilage is not sufficient evidence to conclude that it is consistent with the NF- κ B gene expression signature, since canonical NF- κ B signaling is activated not by increased p65, but by phosphorylation events that induce its translocation to the nucleus (12). Furthermore, the expression of Myf6/MRF4 does not provide sufficient proof of a muscle cell gene expression program in OA cartilage without evidence of Myogenin or MyoD expression. The increased in myosin heavy chain 1 (Myh1) does not reflect true muscle cell differentiation, which can occur in MyoD knockout cells that do not differentiate past the immature myoblast stage unless NF- κ B is inhibited (27). In addition, recent functional studies clearly show that canonical NF- κ B activation antagonizes the muscle cell differentiation program by multiple mechanisms (28).

Interrogation of profiling sets of data such as those provided by Poulet et al. (8) will help other researchers in the field to further our understanding of the OA disease process and find targets that are common across the models or unique to specific models that may reflect the diversity of human OA. Together, the findings from these studies indicate that the design of the experimental model and its outcome depend upon the questions asked and that combinatorial analyses will not only provide insights into mechanisms of human disease, but also into how to target therapy once well-defined clinical cohorts are available.

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