Research Focus

Mouse models of osteoarthritis provide new research tools

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The options for pharmaceutical intervention of osteoarthritis are limited. There is therefore a crucial need for animal models of osteoarthritis that can be used as research tools for improving our understanding of the disease and for discovering and testing new treatments. Recently, a new mouse model of osteoarthritis was described in which the mice are unable to produce a bone morphogenetic protein (BMP) receptor specifically in joints. A comparison of this model to other models with different etiologies is presented. The findings suggest that all of the models could be linked molecularly.

Crucial need for new models of osteoarthritis

Osteoarthritis is characterized by the erosion of cartilage tissue covering the surface of joints, leading to pain and restricted motion and mobility. At present there are no treatments that can reverse the loss of joint tissue that occurs during the progression of the disease. Although the etiology of the disease is unknown, there are multiple factors that could be involved, including genetic predisposition, lifestyle (e.g. obesity or trauma) or anatomy (misalignment of joints). The major recourse for afflicted patients is treatment with non-steroidal anti-inflammatory (NSAIDs), which are prescribed for pain relief. However, Vioxx®, a widely prescribed NSAID, was recently removed from the market after it was discovered that it increases the risk of myocardial infarctions and strokes [1]. This, together with the threat of removing similar NSAIDs from the market, leaves a crucial need for the development of new treatments for this debilitating and prevalent disease.

Tissue-specific gene-deletion model of osteoarthritis

A new animal model of osteoarthritis was described recently that was developed originally to improve understanding about the role of bone morphogenetic protein (BMP) in cartilage development [2]. The model was developed using mice in which the gene encoding BMP receptor type 1a (*Bmpr1a*) was flanked by Cre-loxP sites [3]. Cre-loxP sequences are recognized by the bacteriophage P1-derived enzyme cre-recombinase, which can delete all the DNA positioned between the Cre-loxP sites [4]. Tissue-specific gene depletion of this BMP receptor was accomplished by directing cre-recombinase expression to developing joints using a promoter of the gene encoding growth differentiation factor 5 (cartilage-derived morphogenetic protein 1) (*Gdf5*) [2]. When the *Cre-Gdf5* mice (that produce cre-recombinase where *Gdf5* is expressed) were mated with the *Bmpr1a* mice (that possess the Cre-loxP sites), a new strain of mouse was created that was unable to produce BMP receptor type 1a (termed *Cre-Gdf5/Bmpr1a*^{floxP}) selectively in developing joints. These mice displayed retention of webbing between some digits, lack of formation of some joints in the ankles, and premature osteoarthritis in other joints compared with normal wild-type mice. These data clearly show that BMP signaling is required for the maintenance of joint tissue integrity and strongly imply that defects in BMP function are linked molecularly to post-natal joint destruction.

Double knockout model of osteoarthritis

Other models of osteoarthritis have been described that share many features of the *Cre-Gdf5/Bmpr1a*^{floxP} mice but have targeted deletions (knockout) of the gene encoding biglycan (*Bgn*) or the gene encoding fibromodulin (*Fmod*). These mice, like the *Cre-Gdf5/Bmpr1a*^{floxP} mice, developed earlier and more-severe osteoarthritis compared with normal wild-type littermates [5]. When the expression of both *Bgn* and *Fmod* was prevented simultaneously (double knockout) the incidence and severity of osteoarthritis increased and was more than the sum of that occurring in each of the single knockout animals, which suggests that *Bgn* and *Fmod* act synergistically to maintain joint integrity [5]. *Bgn* and *Fmod* belong to a family of genes that encode small leucine-rich-repeat proteoglycans (SLRPs) or leucine-rich proteoglycans (LLRs). These proteoglycans can bind to collagen and growth factors such as transforming growth factor β (TGF-β). TGF-β and BMP are part of a large superfamily of proteins whose members are related structurally and functionally [6]. In a recent study using skeletal cells from normal and *Bgn* knockout mice, BMP-2 and BMP-4 binding to cells and subsequent downstream signaling was defective in the absence of biglycan (in knockout animals) [7]. These data suggest that biglycan facilitates the action of BMP, providing a molecular link between these two 'phenocopies' of osteoarthritis. In both the *Bgn–Fbn* double knockout mice and *Cre-Gdf5/Bmpr1a*^{floxP} mice, the osteoarthritis was manifested as non-inflammatory fibrillation and subsequent thinning of the articular cartilage joint surfaces. Moreover, decreased safranin O staining (used to measure the proteoglycan GAG chains)

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in the *Cre-Gdf5/Bmpr1a*^{floxP} mice [2] mimicked what was observed in the *Bgn–Fbn* double knockout mice. Further evidence to support the concept that these two animal models of osteoarthritis are linked comes from analysis of the localization of biglycan, fibromodulin and GDF5. All are present in high levels in the articular cartilage of developing joints [2,8].

Genetic animal models of osteoarthritis: similar or different?

Although both the *Bgn–Fbn* double knockout and the *Cre-Gdf5/Bmpr1a*^{floxP} mouse models of osteoarthritis share similarities they are not identical. *Cre-Gdf5/Bmpr1a*^{floxP} mice have delayed apoptosis of the webs between certain digits and selective joint fusion abnormalities that are not observed in *Bgn–Fmod* knockout mice. Unlike *Gdf5/Bmpr1a*^{floxP} mice, *Bgn–Fmod* knockout mice first develop an abnormal gait (outstretched leg), temporally leading to ectopic calcification within the tendons that accompanies a decrease in 'stiffness' (a biomechanical measurement of tendon strength). Manifestations of osteoarthritis in these *Bgn–Fmod* knockout mice follows at 1–2 months of age in a timeframe that is similar to the *Cre-Gdf5/Bmpr1a*^{floxP} mice. A major difference between the two models is that in the *Cre-Gdf5/Bmpr1a*^{floxP} mice the depletion of BMP signaling is limited to joints, whereas in the *Bgn–Fmod* knockout mice there is a total depletion of the two proteoglycans throughout development and in all tissues. Some plausible explanations for the observed differences between the phenotypes of the two different osteoarthritis models might also be related to unique compensatory changes resulting from the absence of each protein. Indeed, skeletal cells isolated from the bones of *Bgn* knockout mice have increased expression of the related SLRP decorin, compared with normal wild-type animals [7]. Interestingly, mRNA profiling using microarray technology has shown that 12% of all expressed genes were different between the normal and the biglycan-deficient skeletal cells and that some but not all of the changes in gene expression resulting from the absence of biglycan could be 'rescued' by BMP treatment [17]. This observation suggests that a functional relationship exists between BMP and the matrix proteoglycan biglycan, which provides further evidence that the two models of osteoarthritis are linked molecularly. It is not known whether a similar mRNA and protein compensation (e.g. at the BMP receptor level) takes place in the *Cre-Gdf5/Bmpr1a*^{floxP} mouse model.

There are several questions that remain to be answered about the cell and molecular similarities of the two aforementioned mouse models and how they might mimic human osteoarthritis. First, it would be important to know if added mechanical stimulation from impact (e.g. running) would exacerbate the osteoarthritis in the *Cre-Gdf5/Bmpr1a*^{floxP} mice as it does in *Bgn–Fmod* knockout mice. Similarly, the adult *Cre-Gdf5/Bmpr1a*^{floxP} mice have impaired ability to grasp and limited range of motion features that remain to be examined in the *Bgn–Fmod* knockout mice. Analysis of molecular markers produced by the affected cartilage [e.g. cartilage oligomeric matrix protein (COMP), aggrecan or type II collagen] in the two models also needs to be evaluated more thoroughly.

Other genetic mouse models of osteoarthritis

The *Cre-Gdf5/Bmpr1a*^{floxP} mice and *Bgn–Fmod* knockout mice are not the only mouse models of osteoarthritis. Other lines that acquire premature osteoarthritis have mutations or deletions of genes encoding several matrix and associated proteins, including type II, IX and XI collagen [9–11], membrane type 1 matrix metalloproteinase (MT-1-MMP) [12] and the α1 integrin subunit [13]. Interestingly, many of the models (including the STR/ort model [14] of unknown etiology) exhibit a dramatic loss of proteoglycans (determined by safranin O staining), which indicates that the proteoglycans could be at the molecular 'crossroads' and crucial downstream targets for factors that affect the development of osteoarthritis.

Recently, mouse lines were created with a targeted deletion of the catalytic domain of ADAMTS5 [a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif 5]. ADAMTS5 is an enzyme that degrades aggrecan, an abundant large proteoglycan found in cartilage [15,16]. ADAMTS knockout mice were phenotypcially normal but resisted osteoarthritis induced by surgery or inflammation. These data suggest that a new approach for the pharmaceutical intervention of osteoarthritis would be to inhibit the active site of ADAMTS5. Taken together, mouse models that either resist osteoarthritis or prematurely acquire osteoarthritis can be useful research tools to understand the molecular basis of osteoarthritis and for designing new treatments for the disease.

Concluding remarks

The key advantage of genetically engineered mouse models of osteoarthritis compared with other surgically induced models is that the molecular etiology is known and that the severity and incidence of osteoarthritis can be controlled. In summary, new lines of mice that acquire premature osteoarthritis will be important new tools to: (i) uncover potential molecular markers for the onset of the disease; and (ii) understand the role of gender and other environmental factors in regulating the occurrence of osteoarthritis. In all cases, knowing more about the molecular basis of osteoarthritis will be crucial for designing and testing new pharmaceutical and nutriceutical ways to treat diseased joint surfaces.

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Lord of the rings – the mechanism for oxidosqualene:lanosterol cyclase becomes crystal clear

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The enzyme oxidosqualene:lanosterol cyclase (OSC) represents a novel target for the treatment of hypercholesterolemia. OSC catalyzes the cyclization of the linear 2,3-monoepoxysqualene to lanosterol, the initial four-ringed sterol intermediate in the cholesterol biosynthetic pathway. OSC also catalyzes the formation of 24(*S*),25-epoxycholesterol, a ligand activator of the liver X receptor. Inhibition of OSC reduces cholesterol biosynthesis and selectively enhances 24(*S*),25-epoxycholesterol synthesis. Through this dual mechanism, OSC inhibition decreases plasma levels of low-density lipoprotein (LDL)-cholesterol and prevents cholesterol deposition within macrophages. The recent crystallization of OSC identifies the mechanism of action for this complex enzyme, setting the stage for the design of OSC inhibitors with improved pharmacological properties for cholesterol lowering and treatment of atherosclerosis.

Inhibition of cholesterol synthesis as a therapeutic target for hypercholesterolemia and atherosclerosis

Since the elucidation and characterization of cholesterol biosynthesis by Konrad Bloch and his colleagues >50 years ago [1], who would have guessed that this complex cascade of 25 enzymatic reactions would continue to teach us new tricks? Probably the best-understood enzyme involved in this pathway is hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, which is the target of the statin class of cholesterol-lowering drugs [2] (Figure 1). Statins have revolutionized the treatment of hypercholesterolemia and cardiovascular disease, and recent landmark clinical trials of statin treatments have revealed significant reductions in cardiovascular mortality and morbidity that are associated with lowering cholesterol levels [3]. Statins are well tolerated, although, theoretically, reductions in the levels of non-sterol intermediates (e.g. isoprenoids and coenzyme Q) synthesized through the 'second messenger' branch of the cholesterol synthesis pathway (Figure 1), could be associated with adverse clinical events, particularly at high doses of statin [4,5]. Furthermore, as the recommended clinical treatment targets for plasma LDL-cholesterol reduction become lower, many patients will not achieve the recommended low-density lipoprotein (LDL)-cholesterol concentrations with current therapies [3]. This has stimulated the search for, and development of, compounds that inhibit cholesterol biosynthesis but act distal to the 'second messenger' branch pathway, thereby preserving the synthesis of metabolically important non-sterol molecules.

Oxidosqualene:lanosterol cyclase catalyzes the formation of the first sterol in cholesterol biosynthesis

2,3-Oxidosqualene:lanosterol cyclase (OSC; EC 5.4.99.7) is a microsomal enzyme that functions downstream of squalene in the cholesterol biosynthetic pathway (Figure 1).

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