New targets for disease modifying osteoarthritis drugs: chondrogenesis and Runx1

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Osteoarthritis (OA) has recently been defined as a 'whole joint' disease with pathological changes in all tissues, including articular cartilage degradation, subchondral bone thickening, osteophyte formation, synovial inflammation and degeneration of ligaments, and, in the knee, the menisci.[1] OA is a chronic musculoskeletal disease that leads to pain and severe impairment of mobility. OA is the main cause of work incapacity and one of the most common reasons for visiting primary care physicians; its prevalence reaches up to 40% of people over the age of 70.[2] In later stages of OA, patients experience limited mobility and persistent pain, often requiring reconstructive surgery at that time. Although OA is the most prevalent rheumatic disease, affecting 40 million patients in Europe, existing therapies are symptomatic and pursue only pain alleviation with no effect on slowing disease progression.[3 4]

While affecting all structures within a joint, OA has generally been defined as a disease characterised by loss of hyaline articular cartilage. Adult articular cartilage exists as four distinct cellular zones. The superficial zone consists of one to two layers of flattened chondrocytes expressing proteoglycan 4 or lubricin, SRY-box 9 (Sox9), type II collagen, aggrecan, tenascin C and low levels of cartilage intermediate layer protein. Chondrocytes of the intermediate zone are round in appearance, do not express lubricin, but do express higher levels of cartilage intermediate layer protein. The chondrocytes of the radial and calcified zones express markers of chondrocyte differentiation and hypertrophy, such as type X collagen and alkaline phosphatase. Each of the articular cartilage regions is normally maintained throughout adulthood unless stress-related injury, inflammation or a genetic defect leads to the loss of either the signals required to maintain these cells or the signals required to inhibit excessive differentiation of these cells. Disruption or impairment of the signals that inhibit excessive differentiation is believed to be the basis for diseases, such as OA.[5 6] Therefore, much attention is being directed toward studying cellular and molecular mechanisms that regulate chondrogenesis and chondrocyte differentiation.

Research has focused on factors that affect chondrocyte metabolism and on the mechanism of cartilage matrix degradation. This has yielded creative approaches to cartilage protection, including the use of agents active on bone turnover, antibiotics, such as minocycline, which inhibit metalloproteases, and other drugs that nullify the effects of cytokines on cartilage. These types of therapies are termed disease modifying OA drugs (DMOAD) or chondroprotective treatments, because they are focused on preventing the loss or encouraging the regrowth or healing of damaged articular cartilage. Despite advances in our understanding of cartilage metabolism and disease process and the development of new agents and treatment strategies, DMOAD agents have not as yet been added to the therapeutic arsenal. This is generally because such treatments have not demonstrated clear-cut efficacy in human disease. This statement is in itself controversial because a number of treatments have shown possible efficacy. However, no regulatory agency has approved a therapy that is a DMOAD.[3 4 7] Efficient strategies for the development of new OA modifying therapies are therefore essential. These approaches include the identification of those factors that are players in OA and can serve as targets for prompt pharmaceutical treatments.

Importantly, Yano *et al* have identified a new potential DMOAD candidate (TD-198946) with strong chondrogenic activity, tested its efficacy as a DMOAD using an OA animal model and investigated its mechanism of action.[8] These authors propose that the compound exerts its effect by regulating Runx1 expression, which possibly underlies the therapeutic effect of TD-198946 on OA.

To understand this exciting approach retarding cartilage degradation, it is necessary to know some concepts from the embryology, cellular biology and histology of cartilage. Chondrogenesis (the process by which cartilage is formed) is a process that is important for the creation of chondrocytes both during embryogenesis and in adult life. The process begins with the aggregation and condensation of loose mesenchyme. During the early stage of chondrogenesis, the condensing mesenchyme expresses various extracellular and cell adhesion molecules, including type II collagen, N-cadherin, tenescin C, as well the transcription factor, Sox9 (figure 1).[6 9] As the mesenchyme differentiates into chondrocytes, the cells begin to produce an extracellular matrix rich in the mature form of type II collagen and aggrecan. Eventually, these cells begin the process of hypertrophy. Prehypertrophic chondrocytes enlarge slightly and initiate the expression of an inhibitor of hedgehog, a parathyroid hormone-related protein receptor, and also increase expression of alkaline phosphatase and the important regulatory transcription factor Runx2, which aids in chondrocyte differentiation and is required for cartilage mineralisation.[10] As hypertrophy proceeds, the cells continue to enlarge, generate a mineralised matrix, and further enhance their expression of type X collagen, Runx2, and several growth factors that coordinate chondrocyte proliferation and differentiation. Only the most terminally differentiated hypertrophic chondrocytes express the matrix-degrading enzyme metalloprotease 13.[11]

Yano *et al* show that TD-198946 strongly induced in vitro chondrogenic differentiation of progenitors, as evidenced by a marked increase in the chondrogenic markers type II collagen and aggrecan; it did this without promoting hypertrophy (type X collagen and metalloprotease 13).[8]

This interesting balance between chondrogenesis and hypertrophic differentiation may be because TD-198946 exerts its effect through regulating the expression of Runx1, a known inducer of chondrogenic differentiation and a suppressor

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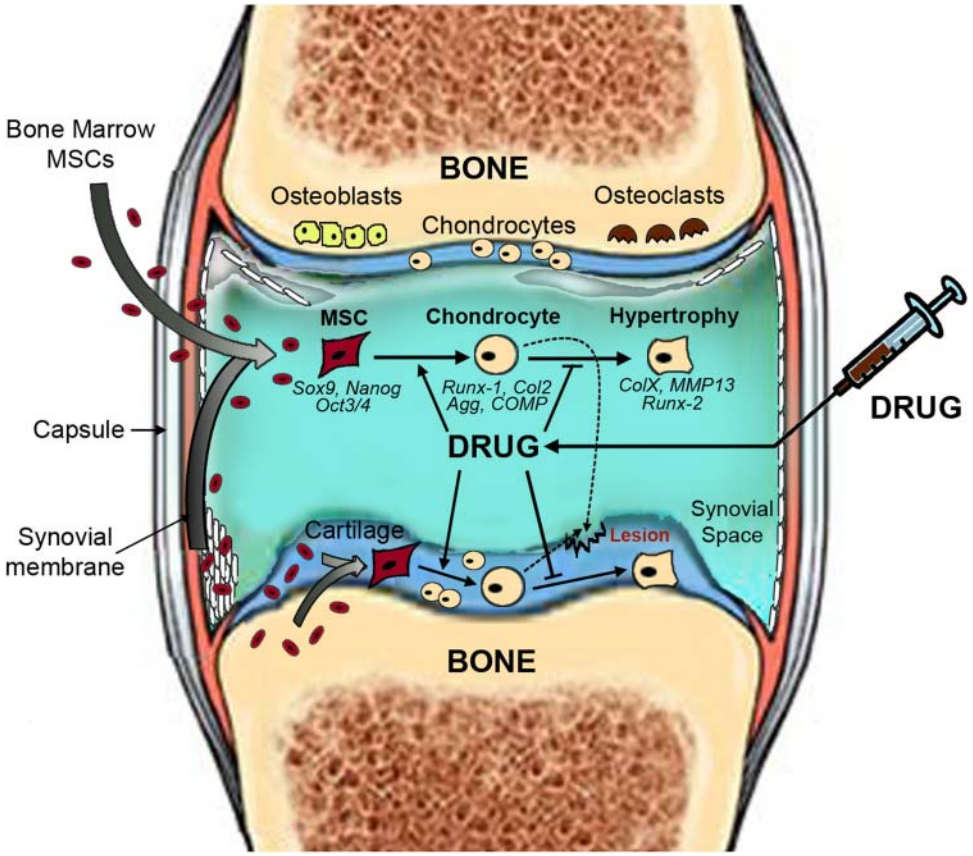

Figure 1 Localisation of mesenchymal cells, the chondrogenesis process in the joint and targets for a new DMOAD: Three locations for MSC have been identified in the OA joint. (a) articular cartilage, (b) synovial fluid and (c) synovial tissue. MSC can reach the sinovium by migration from systemic circulation or from haematopoietic marrow through subchondral bone. Chondrogenesis is the process for creation of chondrocytes. During the early stage in chondrogenesis, MSC expresses such transcription factors as Sox9, Nanog and Oct3/4. As the cells differentiate into chondrocytes, the cells begin to produce an extracellular matrix rich in the mature form of type II collagen (Col2), COMP and aggrecan (Agg) as well as the transcriptor factor Runx1. Eventually, these cells begin the process of hypertrophy. As hypertrophy proceeds the cells continue to enlarge, generate a mineralised matrix and further enhance their expression of type X collagen (ColX), Runx2 and MMP13. Intra-articular administration of drugs increasing the Rux1 expression can induce chondrogenesis and block hypertrophy promoting the repair of damaged articular cartilage. COMP, cartilage oligomeric matrix protein; DMOAD, diseases modifying for OA drugs; MMP13, metalloprotease 13; MSC, mesenchymal stem cell; Sox9, SRY-box 9; OA, osteoarthritis.

of the subsequent hypertrophy.[10] The mammalian Runx protein family comprises three transcription factors: Runx1, Runx2 and Runx3. All Runx proteins are expressed during chondrogenesis; however, experimental results show a distinct role for Runx proteins in chondrogenesis and subsequent chondrocyte maturation.[12 13] Runx1 is highly expressed during chondrogenesis in comparison with Runx2. Overall, the expression of Runx1 remained significantly higher than Runx2 expression during early limb bud cell maturation. Studies further indicate a role for Runx1 during early stages of chondrocyte maturation, whereas Runx2 is important in mediating terminal chondrocyte differentiation.[12] Yano *et al*[8] show that Runx1 is expressed in the superficial and middle zones of adult normal cartilage (mouse and human) above the tidemark, but not in mineralised cartilage; however, its expression is downregulated in OA cartilage. Runx1 expression was restored by TD-198946 treatment, and gain- and loss-of-function experiments revealed that Runx1 mediated the chondrogenic action of TD-198946.[8] Runx1 directly enhanced the promoter activity of COL2A1 through specific binding to a Runx motif.

The potential important role of Runx1 as a target to treat cartilage degradation proposed by Yano *et al*[8] is supported by a recent publication reporting another small molecule, Kartogenin (KGN),[14] with chondrogenesis capacity but with slight effect on hypertrophy and calcification. KGN has the capacity to induce chondrogenesis by regulating the binding of the core-binding factor β subunit (CBFβ) to Filamina A (FLNA) or Runx1. FLNA is an actin-binding protein that cross-links actin filaments and its expression is increased in OA human mesenchymal stem cells (MSCs).[15] The binding of CBFβ to Runx1 induces chondrogenesis; however, its binding to FLNA reduces chondrogenesis. KGN blocks the interaction between FLNA and CBFβ, promoting translocation into the nucleus of CBFβ where it binds to Runx1 and regulates transcription and chondrogenesis. Lineage-specific differentiation in the presence of KGN was confirmed by fluorescent immunostaining of chondrocyte-specific proteins, including type II collagen, Sox9 and aggrecan.[14] These authors suggest that KGN also has DMOAD capacity because the intra-articular administration of KGN, and subsequent histological analysis and grading of the medial tibial plateau, revealed regeneration in the cartilage matrix, as indicated by a decrease in the fibrillations in the superficial and mid-zone of the articular cartilage. In addition, there were significantly lower levels of cartilage oligomeric matrix protein in the mice treated with KGN.

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How do drugs induce chondrogenesis in the joint? To answer this question it is necessary to demonstrate the presence of cells with chondrogenic capacity in joints. It is already known that when a tissue is damaged, repair mechanisms involving stem cells or inflammatory cells are initiated in the compromised tissue or migrate to it through the vascular system. Because articular cartilage is avascular, it must either self-repair or rely on neighbouring tissues for repair. It has been reported that human articular cartilage tissue has cells with chondrogenic capacity that may participate in the repair of cartilage lesions. At the surface, chondrocytes show distinct spatial patterns in single cells, pairs, clusters or strings depending upon the joint type and have the properties of MSCs.[16–19] A loss of chondrocytes expressing high-mobility group protein 2 (HMGB2) in the cartilage superficial zone occurs with ageing,[20] but there is an increase in cells expressing the mesenchymal progenitor cell markers, Stro1 and Notch1 in the chondrocyte clusters appearing during OA.[21] An important point in patients with OA is that their articular chondrocytes show 'phenotypic plasticity' comparable with MSCs undergoing chondrogenesis.[22 23] This group of cells might be a target of TD-198946 to induce chondrogenesis (figure 1). In their animal model, Yano *et al*[8] show that Runx1 is expressed in the superficial and middle zones of adult normal cartilage above the tidemark, but not in mineralised cartilage. In addition, its expression is downregulated in human OA cartilage. Interestingly, expression of Runx1 was higher in TD-198946-treated cartilage than in saline-treated cartilage.[8] However, these results are controversial because other authors have reported higher levels of Runx1 mRNA in human OA cartilage than in normal cartilage.[24]

An additional factor in the repair process involves the synovial membrane as a neighbouring tissue.[25 26] It is possible that cells in the synovial membrane having chondrogenic potential can migrate to damaged cartilage to participate in the active process of cartilage regeneration and repair. In support of this possibility, higher levels of MSCs markers have been found in the synovium and synovial fluid samples of patients with OA than in samples from healthy donors.[26–28] Cells with MSC markers were located in the subintimal zone and around blood vessels in the synovium. Because inflammatory cells found in synovial membranes are derived from haematopoietic marrow and transported through the vascular system and peripheral blood, it is also suggested that MSCs may similarly migrate to OA synovial membranes. The presence of cells expressing MSC markers in the synovial tissues of healthy donors also suggests that in situ proliferation of MSCs in the synovium is possible as well.[26] Finally, it has been reported that canals between the bone marrow and articular cartilage allow migration of MSCs with chondrogenic capacity into the cartilage and synovium of inflamed joints.[23] Drugs could induce chondrogenesis in mesenchymal cells located in the superficial zone of normal and OA cartilage, in cells coming from haematopoietic marrow to the deep zone of cartilage, or in the synovium. The results shown by Yano *et al*[8] in their paper do not provide an answer to this question.

In animal models during the last decade, several molecules have been proven to have a DMOAD effect, and new candidates for DMOAD are emerging every year; however, none of these are indicated to treat OA patients. Some of the more recent candidates are intra-articular injections of antibodies specific for syndecan-4, which is involved in aggrecan cleavage by a disintegrin and metalloproteinase with a thrombospondin type 1 motif-5 (ADAMTS-5), which prevented cartilage destruction in a surgically-induced model of OA.[29] Intraperitoneal injections of an inhibitor of the Hedgehog signalling activated in OA reduced the severity of OA by inhibiting cartilage destruction, possibly by Runx2 regulation, which in turn regulates ADAMTS-5.[30] Subcutaneous injections of recombinant human PTH(1–34) (teriparatide) immediately, or 8 weeks after OA induction, partially reduced the severity of OA by suppressing inappropriate articular cartilage hypertrophy.[31] In general, the major action of these agents is to block activation of the degradation enzyme and/or inhibits aberrant hypertrophy of articular cartilage by targeting signalling molecules involved in articular cartilage catabolism. However, TD-198946 induces chondrogenic differentiation of progenitors, as evidenced by a marked increase in the chondrogenic markers type II collagen and aggrecan, and it does this without promoting hypertrophy. This feature is very important because hypertrophic differentiation is a frequent and undesirable effect observed with conventional chondrogenic agents, including bone morphogenetic proteins, transforming growth factor-β, insulin and insulin-like growth factor (IGF-1).

Although both papers from Yano group[8] and Johnson group[14] suggest chondrogenesis modulation and Runx1 as targets to slow cartilage degradation and OA progression, we must keep in mind that studies of chondroprotective agents using animal models have not provided good models for fully developed DMOAD in humans. In addition, Yano *et al*[8] do not clearly demonstrate evidence of how TD-198946 induces in vivo chondrogenesis. They have also not demonstrated whether the induction of chondrogenesis is responsible for the reduction of joint damage, because MSCs also have anti-inflammatory effects, which could be the cause of joint improvement by an indirect mechanism.

In their paper, Yano *et al*[8] open the possibility that TD-198946 has a preventive effect because administration of the drug, prior to the development of OA, reduces cartilage degradation. This option would be possible only if the joint contains native MSCs with chondrogenic capacity. Although the presence of cells with MSC markers and chondrogenic capacity in normal human cartilage and synovium has been reported,[17 18 21 26] we do not know how preventive chondrogenesis can stop the development of cartilage degradation and initiation of the OA process.

In addition to efficacy, drug safety is a very important point when administering drugs in clinical practices. To satisfy this concern, the side effects of chondrogenesis modulation and Runx1 stimulation must be analysed in detail in animal models and during application in human OA clinical trials. Overexpression of Runx1, Runx2 or Runx3 in non-hypertrophic chondrocytes led to important skeletal abnormalities overall in the vertebral column.[12] These results indicate that the expression of these genes at precise times during skeletal development and chondrocyte differentiation is important, and that each gene must have a distinct role in the differentiation of chondrocytes. Obviously, avoiding systemic administration could reduce this type of side effect. However, even if TD-198946 or KGN is delivered by intra-articular injection, adequate preclinical studies must be performed to assess side effects. Another side effect described in animal models from overexpression of Runx1 or Runx2 is ectopic mineralisation.[12] Intra-articular administration of drug might promote the calcification of the meniscus or synovium. These side effects were not analysed in the study commented on in this editorial.[8]

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In conclusion, because cells with chondrogenic capacity have been identified in healthy and diseased articular tissues (cartilage and synovium) and appear to retain at least some potential to regenerate/repair cartilage in vivo, compounds with chondrogenic capacity are a good option for development of a new DMOAD. Transcription factor Runx1 appears to be an ideal target to stimulate chondrogenesis in the joint. Drugs with the capacity to modulate Runx1 activity, such as TD-198946 and KGN, when administered directly into the joint space, successfully stopped the degeneration of articular cartilage in a surgically-induced OA mouse model. Rheumatologists need DOMOADs to treat OA patients; now is the time to confirm these exciting preclinical results by properly conducted clinical trials.

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