Joint homeostasis, restoration, and remodeling in osteoarthritis

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Osteoarthritis is the major cause of joint failure. The outcome of the disease process is determined by complex interactions between cells and molecules steering homeostasis, destruction, restoration, and remodeling. The articular cartilage has a limited restoration and repair capacity. Genetic studies in humans and the development of mouse models have identified the role of signaling pathways that are important for skeletal development in the postnatal biology and pathology of articular cartilage. These include bone morphogenetic protein, transforming growth factor beta, fibroblast growth factor, wingless-type signaling, and their respective antagonists such as noggin and frizzled related protein. The synovium is prone to inflammation and emerging evidence suggests that innate and adaptive immune responses are important. Bone and cartilage form a biomechanical unit; stiffer bones might impair cartilage homeostasis. The biology of frizzled related protein provides a basis for the hypothesized inverse relationship between osteoarthritis and osteoporosis.

Key words: bone morphogenetic protein; bone; cartilage; frizzled related protein; osteoarthritis; synovium; wingless-type (WNT).

INTRODUCTION

The impact and outcome of physiological and pathological processes in the joint and bone are determined by molecular signaling pathways that regulate tissue homeostasis, restoration, and remodeling.1 Homeostatic processes ensure the adaptive maintenance of tissue integrity and function. This can fail in case of strain or direct injury, which result in tissue damage. Tissue responses to damage aim to restore function, integrity, and homeostasis. Ideally, this is achieved by tissue restoration. However, in many cases, damage instead leads to tissue remodeling with either the formation of...
a surrogate tissue (repair) or new tissue. The remodeling process does not result in full restoration of integrity and homeostasis and might therefore fail in the long term, with progressive loss of function as a consequence.

The osteoarthritic diseases are a group of disorders characterized by gradual failure of the joints, leading to damage, pain, and disability. The primacy of cartilage damage versus excessive stress in the bone–cartilage biomechanical unit as the driving mechanism for osteoarthritis (OA) is still debated. It is clear, however, that the healthy joint requires a fine-tuned balance between molecular signals that regulate homeostasis, damage, restoration, and remodeling. This balance is determined at the level of the individual cells, of the tissue architecture, and of the interactions between different tissues in the joint organ, e.g. articular cartilage, synovium, and bone.

Different factors appear to impair maintenance of homeostasis in a joint that has been damaged or strained. First, the articular cartilage is a very specialized tissue, the function of which is critically dependent on its physicochemical properties. It is rich in nonvascular, paucicellular extracellular matrix, which might explain its limited potential for regeneration and repair. Second, adaptive responses in the bone with which the articular cartilage is forming a biomechanical unit might strengthen the bone component but thereby inappropriately impair cartilage homeostasis and function. Third, articular chondrocytes can produce a number of proinflammatory cytokines and tissue-destructive enzymes. Under physiological circumstances these might play a role in normal tissue homeostasis and turnover. In situations of damage, they could inappropriately enhance tissue destruction.

Increasing evidence supports the hypothesis that tissue restoration and remodeling in different forms of arthritis are regulated by signaling pathways that are also involved in the development of cartilage and bone. In particular, transforming growth factor beta (TGFβ), bone morphogenetic protein (BMP), wingless-type (WNT), and fibroblast growth factor (FGF) signaling have been studied in OA. Here, we focus on three important questions and their potential clinical implications: (1) What is the role of these signaling pathways in homeostasis, restoration, and remodeling of the articular cartilage? (2) How do these pathways regulate and influence the interactions between cartilage, bone, and synovium in the joint? (3) Are these pathways involved in new tissue formation in OA?

**ACTIVATION OF SIGNALING PATHWAYS CRITICAL FOR JOINT DEVELOPMENT IN POSTNATAL ARTICULAR CARTILAGE**

In-vitro cell culture experiments, cartilage explant analysis, in-vivo genetic models (Table 1), and gene transfer protocols, as well as genetic studies in humans, support a role for the above-mentioned signaling pathways in the biology and pathology of articular cartilage. The wealth of data generated also highlights the complexity of the processes involved. In addition, these signaling pathways might have a wide range of effects in vivo, depending on factors such as target cell type and differentiation. For instance, different members of the TGFβ superfamily, such as TGFβs, BMPs, and related cartilage-derived morphogenetic proteins (CDMPs) have anabolic effects on chondrocytes (for recent review, see ref. 7). However, these molecules should be considered as pleiotropic morphogens, growth factors, and cytokines with diverse effects on distinct cell types. Indeed, TGFβ is not only a chondrogenic growth factor but also an immune-regulating cytokine and a potent stimulus of fibrosis. BMPs were originally identified as proteins that can induce a cascade of ectopic endochondral bone
formation in vivo\textsuperscript{10,11} but are also critical during developmental patterning.\textsuperscript{12} The respective roles of the above-mentioned pathways in cartilage biology and OA is most strongly supported by data from in vivo genetic mouse models using either loss or gain of function approaches.

**Bone morphogenetic proteins**

CDMP1 was originally identified from a chondrogenic extract of articular cartilage.\textsuperscript{13} In the mouse, its homolog growth and differentiation factor 5 (Gdf5) is expressed in the joint interzone during development.\textsuperscript{13} A spontaneous mutation in this gene leads to joint fusions in mice.\textsuperscript{14} Similar phenotypes are found in human chondrodysplasias caused by mutations in the CDMP1 gene.\textsuperscript{15–17} Of specific interest, a genetic association between OA and polymorphisms in the regulatory region of CDMP1 has recently been described.\textsuperscript{18} The protein is present in normal human adult articular cartilage.\textsuperscript{19} Its expression is mostly restricted to the superficial cartilage in normal joints, whereas in OA cartilage the expression domain extends into damaged areas.\textsuperscript{19} Recombinant CDMP1 increases proteoglycan synthesis in adult articular cartilage explants that have been partially matrix depleted by mild trypsin treatment.\textsuperscript{20} The in-vivo role of Gdf5 in animal models of OA, however, remains to be studied.

The developmental expression of Gdf5 in the joint interzone was used to generate a joint-specific Bmp-receptor (Bmpr) knockout mouse.\textsuperscript{21} Heterozygous Bmprla\textsuperscript{+/−} mice, engineered to express a Cre recombinase in the Gdf5 locus (Gdf5\textsuperscript{Cre/Cre};Bmprla\textsuperscript{+/−}), were crossed with mice that carry a floxed Bmprla allele (Gdf5\textsuperscript{+/+};Bmprla\textsuperscript{loxP/loxP}). Expression of the Cre gene leads to cell-specific deletion of the floxed Bmprla gene. Therefore, the Gdf5\textsuperscript{+/+};Cre;Bmprla\textsuperscript{loxP/loxP} conditional knockout mice have no BMPRLa in the progeny of cells that expressed Gdf5 during development. These mice show some mild developmental defects (short ears, soft-tissue syndactyly between digits 1 and 2, and tarsal joint ankylosis), but more importantly they developed rapid cartilage loss after birth.\textsuperscript{21} These data suggest that BMP signaling through BMPRLa is essential to maintain cartilage homeostasis.

Our observations in noggin (Nog) haploinsufficient mice (Nog\textsuperscript{+/+}/LacZ) provide further support for this hypothesis.\textsuperscript{22} NOG is a secreted antagonist of BMP signaling that binds to different BMP ligands and is expressed in the articular cartilage.\textsuperscript{22} Nog

| Table 1. Genetic models showing a role for developmental signaling pathways in OA. |
|---------------------------------|-----------------|
| Bone morphogenetic protein signaling | Reference |
| Tissue-specific Bmprla knockout mice | 21 |
| Nog haploinsufficient mice | 22 |
| Transforming growth factor beta signaling | |
| TgfbrII dominant negative mice | 28 |
| Smad3 knockout mice | 29 |
| Wingless-type signaling | |
| Frzb knockout mice | 48 |

Bmprla, bone morphogenetic protein type Ia receptor; Frzb, frizzled related protein; Nog, noggin; Smad3, Smad family member 3; TgfbrII, transforming growth factor beta type II receptor.
knockout mice are embryologically lethal whereas the haploinsufficient mice are viable but with a +50% reduction in Nog expression levels.22,23 Reduced NOG levels in Nog<sup>+/−</sup>/LacZ mice protect the articular cartilage in a mouse model of inflammatory joint destruction. Reduction in NOG enhanced BMP signaling in the articular cartilage, as demonstrated by immunohistochemistry for phosphorylated SMAD1/5, a downstream mediator in the activated signaling cascade. By contrast, overexpression of NOG in wild-type mice in this model increased cartilage damage, probably by reducing BMP activity.22

These data from genetic mouse models of BMP signaling are further corroborated by a number of in-vitro and in-vivo studies, in particular on BMP2 and BMP7 (for review, see ref. 7). In addition, proinflammatory cytokines such as interleukin-1 (IL1) up-regulate BMP2 in chondrocytes and synoviocytes.24,25 Intra-articular injections of BMP2 in the mouse knee have been used to assess the effect of this BMP on articular cartilage in vivo. BMP2 stimulates proteoglycan synthesis in normal knees but cannot do this in a model of destructive arthritis.26 Injection of BMP2 also triggers osteophyte formation (see below).

These combined findings highlight the potential role of BMPs in the maintenance of the chondrocyte phenotype and the integrity of the postnatal joint surface. Not surprisingly, BMPs have been suggested for tissue restoration or repair therapeutic approaches.27 The use of growth factors for tissue engineering approaches is beyond the scope of this chapter, but the delicate in-vivo balance between BMPs, their antagonists, and their anabolic effects resulting in new tissue formation suggest that this approach will require careful design and optimal control.27

Transforming growth factor beta

TGFBs – members of the same superfamily – can also play a role cartilage biology and OA. Skeletal tissue-specific overexpression of a dominant negative TGFB type II receptor (TGFBRII), thereby effectively neutralizing TGFB signaling, leads to an OA-like phenotype with hypertrophy and terminal differentiation of chondrocytes.28 A similar phenotype is seen in mice deficient in Smad3, a TGFB-associated intracellular signaling molecule.29 Smad3<sup>−/−</sup> mice develop an OA-like disease, characterized by progressive loss of articular cartilage, surface fibrillation, formation of large osteophytes, upregulation of type X collagen, and decreased proteoglycan synthesis at the age of 6 months.

Intra-articular joint injections of TGFB1 have been used to study the in-vivo effects of TGFBs on cartilage metabolism and potential interactions with IL1. Following TGFB1 injection, proteoglycan synthesis rises steadily and the response is maintained for 20 days. Stimulation of endogenous TGFB or BMP production and/or upregulation of receptors are hypothesized as underlying mechanisms for this observation. Remarkably, TGFB1 counteracts the IL1-induced suppression of proteoglycan synthesis whereas BMP2 does not.30–34 Of interest, the effects of TGFB on articular cartilage appear age dependent.35

Wingless-like family

The WNT family consists of over 15 secreted glycoproteins with diverse functions in development and postnatal life. WNT signaling is crucial for joint and bone development.36–38 As with CDMP1, the strongest evidence supporting a role for this pathway in OA has come from human genetics. Polymorphisms in frizzled related protein
(FRZB), a secreted antagonist of WNT signaling\textsuperscript{39–41}, have been associated with OA.\textsuperscript{42–47} Like CDMP1, FRZB was originally identified from a chondrogenic extract of articular cartilage and found to be expressed in developing skeletal elements.\textsuperscript{39} Our group has developed Frzb\textsuperscript{−/−} mice.\textsuperscript{48} The mice do not show apparent developmental abnormalities in the skeleton. However, in different models of cartilage damage, proteoglycan loss is significantly enhanced as compared to wild-type mice. Cartilage damage is associated with increased WNT signaling and matrix metalloproteinase-3 (Mmp3) expression. In addition, FRZB can also directly inhibit MMP activity\textsuperscript{48,49}, providing a second mechanism for an increased incidence of OA in patients with FRZB polymorphisms.

The role of WNTs in postnatal cartilage biology is certainly complex. Our data in the Frzb\textsuperscript{−/−} mice suggest that WNTs would contribute to cartilage damage and hence OA development. However, Dell Accio et al also demonstrated that mechanical injury to cartilage explants results in downregulation of Frzb and upregulation of WNT target genes like axin2. Activation of WNT signaling also upregulates type II collagen and aggrecan expression in their system.\textsuperscript{50}

**Fibroblast growth factors**

FGFs and their receptors (FGFRs) play important roles in multiple biological processes, including mesoderm induction and patterning, cell growth and migration, organ formation, chondrogenesis, and growth.\textsuperscript{51,52} Mechanical injury to cartilage explants results in activation of the extracellular regulated kinase (ERK) mitogen-activated protein kinase (MAPK) pathway. This is mediated by FGF2.\textsuperscript{53} A similar activation cascade was identified in mechanically loaded articular cartilage.\textsuperscript{54} Interestingly, FGF2 is bound in the pericellular chondrocyte matrix to perlecan, suggesting that the trapped growth factor is acting as a ‘structural’ protein.\textsuperscript{55} However, as for WNTs, the effects of FGFs in cartilage and OA are far from clear as FGF2 treatment of cartilage explants results in enhanced expression of MMPs and of tissue inhibitors of MMP (TIMPs).\textsuperscript{53}

**Clinical implications**

The biology of articular chondrocytes in health and disease should guide the development of therapeutic plans to treat or prevent OA. Pharmacological interventions in a later stage of the disease, e.g. when symptoms are already present, might have missed an important window of opportunity to revert the ongoing process of tissue damage and loss of function. Hence, treatments with MMP inhibitors or growth factors are not likely to cure the disease at this stage and at best limit further damage and stimulate repair, not restoration. By contrast, prevention of disease could be an attractive alternative, albeit difficult to achieve. Early recognition of patients at risk might include evaluation of lifestyle risk factors, such as overweight and lack of exercise, as well as genetic profiling. A number of polymorphisms have been identified and important associations have been convincingly replicated in the last couple of years. The identification of high-risk patients might not only allow developing lifestyle measures but could also lead to preventive treatments in an earlier disease stage. This would result in a risk reduction approach not unlike current practice in the prevention and treatment of osteoporosis. However, toxicity of treatments with growth factors as well as enzyme inhibitors might be important limiting factors, particularly in elderly patients who require a long-term therapy. Better understanding of different types of OA, including their specific genetics, might not only help us to better identify subgroups of
patients but also to specifically develop local treatment strategies that would not bear the disadvantages of systemic treatment.

**TISSUE INTERACTIONS IN THE JOINT ORGAN**

Collaboration between different tissues within the joint organ is necessary for normal movement. These include articular cartilage, bone, synovium, ligaments, tendons, and eventually menisci. Not surprisingly, all the tissues of the joint organ can play a role in OA.

**Inflammation in the synovium**

Synovitis and joint effusions have gained increased attention in the last years as they are associated with pain and swelling and therefore directly clinically relevant. A classic hypothesis suggests that the release of factors from cartilage and bone triggers nonspecific inflammation in the synovium. The articular cartilage is resistant to vessel invasion and cellular infiltration. Therefore, any inflammatory reaction in the joint will find its way into the tissue that is most prone to inflammation and that can potentially transform in a secondary lymphoid organ-like tissue: the synovium. Under physiological circumstances, the synovium is a thin tissue that consists of a pseudoepitheloid lining layer with synovial fibroblasts, macrophages, and a loose connective tissue in the sublining zone. Different factors can stimulate angiogenesis and a massive inflammatory cell infiltrate. Joint inflammation with specific tropism for the synovium has recently been put forward in the context of the existence of a synovio-enthesal complex in spondyloarthritis, and this concept can easily be adapted to OA. From this point of view, synovitis can be considered a common final pathway in a tissue that is easily primed for innate immune responses triggered by cartilage damage. However, synovitis in OA patients might be driven by specific antigens and also involve adaptive immunity. Recent work has demonstrated the existence of clonal B-cell populations in OA synovium, pleading against the nonspecific nature of inflammation in OA. Cytokines and tissue-destructive enzymes produced by the synovium can contribute to cartilage destruction. In addition, the synovium can be the source of growth factors that play a role in joint remodeling (see below). However, recent MRI data suggest that synovitis and effusions contribute to joint symptoms rather than to cartilage damage.

**The bone–cartilage biomechanical unit**

Interactions between cartilage and bone are of particular interest from a biomechanical point of view. An inverse clinical relationship between OA and osteoporosis has been hypothesized but remains controversial. Our observations in Frzb−/− mice provide evidence that WNT signaling and its antagonists could present a molecular mechanism for this relationship. In addition to increased cartilage damage in induced models, Frzb−/− mice also have increased cortical thickness, bone mineral density, and content as compared to wild-type littermates. Although at first sight the differences appear fairly limited, the increase in cortical thickness strongly affects the stress–strain relationship in the bone. Therefore, the long bones of Frzb−/− mice are stiffer than those of their wild-type littermates. Increased stiffness is likely to alter the load in the
articulate cartilage providing a third mechanism by which functional polymorphisms in FRZB can play a role in OA.

Frzb−/− mice do not show differences in trabecular or subchondral bone density. Additional research is therefore required to identify factors that lead to increased subchondral bone thickness and thereby cause or accelerate OA. One potential candidate is the FRZB-related secreted frizzled related protein 1 (sFRP1) gene. In line with current concepts on the role of subchondral bone in OA pathology, it seems likely that local or systemic bone remodeling to strengthen the bone might have adverse effects on the cartilage by disturbing the cartilage–bone biomechanical unit.

Clinical implications

The critical involvement of synovitis and joint effusion in pain in a number of OA patients supports the use of anti-inflammatory drugs for their management. As these drugs have significant toxicities, their use should be limited to those patients in whom they are undoubtedly beneficial. Better identification of patient subgroups is therefore essential. The current evidence supporting a role for adaptive immunity in inflammatory changes in OA further strengthens the concept that different subgroups of patients should be distinguished. Theoretically at least, immune modulating or even biologicals could be considered for a number of patients. Again, it is important to consider the potential toxicity. Biomarker development and analysis, as well as further studies of synovium biology in OA patients, are likely to enhance our current knowledge and might have an important impact on therapeutic approaches in the years to follow.

The role of FRZB in the balance between cartilage and bone sheds new light on the important question of exercise needs in the prevention of OA. Frzb−/− mice show increased periosteal new bone formation after cyclic loading in vivo. This will further increase the stiffness of the long bones and compromise normal cartilage biology. However, molecular evidence suggests that a certain amount of loading will stimulate extracellular matrix synthesis and strengthen the cartilage. Nevertheless, excessive exercise, in particular in genetically predisposed individuals, could increase stiffness in the bone–cartilage unit and contribute to the development of OA. Again, identification of biomarkers and genotype analysis could be important means to individualize patient profiles and define both nonpharmacological and pharmacological strategies.

TISSUE REMODELING: OSTEOPHYTE FORMATION

Osteophytes are a characteristic manifestation of remodeling in joints affected by OA. New cartilage and bone formation appear to arise from the anatomical zone where the synovium overlies the bone in a process that recapitulates endochondral bone formation during development and growth. In general, there is no convincing evidence that osteophytes contribute to the signs and symptoms of the disease. Rather, they might represent a stabilizing effort in an already damaged joint in order to deal with load and strain. From this point of view, osteophytes would be considered (ectopic) repair efforts rather than remodeling contributing to pathology. This type of new tissue formation in the joint might therefore be different from that seen in the spondyloarthritides, in which progression towards ankylosis typically results in loss of joint function.

Not surprisingly, molecular signaling pathways such as BMPs and TGFBs also appear to play a role in osteophyte formation. Different groups have studied the presence of
BMPs in human osteophytes. Three different types of bone formation in the growing osteophyte are observed: endochondral and membranous from the periost, and from the endosteum. Immunohistochemistry demonstrated BMP2 in both fibrous matrix and osteoblasts. BMP3 was found in osteoblasts and osteoclasts; BMP6 in osteocytes and osteoclasts; and BMP7 in hypertrophic chondrocytes, osteoblasts, and osteocytes. Nakase et al demonstrated BMP2 in fibroblastic mesenchymal cells, fibrochondrocytes, chondrocytes, and osteoblasts both at the mRNA and protein level. BMP and TGFβ signaling in osteophyte formation have been studied more mechanistically in mouse models of OA. Injection of recombinant BMP2 or TGFβ into healthy murine knees stimulated osteophyte formation. Interestingly, osteophytes induced by BMP2 injection were found predominantly in the regions where the growth plate met the joint space, whereas TGFβ-induced osteophytes originated from the border between cartilage, bone and synovium.

Synovial macrophages appear critical in this process as osteophyte formation induced by TGFβ was reduced after depletion of macrophages. The number of BMP2- and BMP4-positive cells in these experiments also decreased on deletion of the macrophages. Osteophyte formation in papain-induced arthritis can be inhibited by adenoviral overexpression of both BMP and TGFβ antagonists. Again, expression of BMP2 and BMP4 in this model was markedly increased in the synovium.

Of clinical interest, one type of osteophyte in OA might be different in nature. Recent data obtained with MRI imaging suggest that OA of the interphalangeal joints is closely associated with ligaments and their entheseal insertions. In these patients, inflammatory episodes can be very painful and often result in tissue damage with osteophyte formation. Sometimes, the radiographic changes in the joints can be difficult to distinguish from those seen in patients with psoriatic arthritis and distal interphalangeal joint involvement. As joint destruction and remodeling in these joints are associated with loss of function, osteo- and enthesophyte formation can be considered a potential therapeutic target. As indicated from a mouse model of ankylosis, inhibition of BMP signaling might be useful for these patients.

**SUMMARY**

Osteoarthritis is a leading cause of morbidity. Current therapeutic approaches are largely insufficient to prevent initiation and progression of disease. OA is a disease of the joint organ and involves articular cartilage, subchondral and cortical bone, synovium, ligaments, tendons, and their insertions. Genetic studies in humans have identified new molecules that are likely to play a role in the complex pathology of these diseases. Genes identified, like CDMP1 and FRZB, are involved in signaling cascades that are important during skeletal development. The use of genetic mouse models further corroborates their importance in OA. Most research questions have addressed the role of signaling pathways in cartilage. However, accumulating evidence suggests that tissue interactions, in particular between cartilage and bone, are critically involved in OA. From this point of view, our observations in Frzb knockout mice demonstrate that a single molecule might influence both cartilage and bone in OA development. In addition, the biology of FRZB provides a molecular mechanism for the longstanding hypothesis that OA and osteoporosis show an inverse clinical relationship. Major challenges for the clinical research community lie ahead. Molecules and signaling pathways identified have a broad range of functions in the body and interference with these pathways will require a specific, targeted approach. Therefore, local therapies might become as important as systemic interventions.
**Practice points**

- The role of developmental signaling pathways in OA:
  - Genetic studies have identified specific polymorphisms associated with OA. Identification of patients at risk may become increasingly important to adjust preventive lifestyle and pharmacological strategies
  - The biology of growth factors is complex and specific targeted therapeutics need to be developed to prevent significant toxicity
  - Further progress in our understanding of OA must come from the combination of human genetics and animal models of the disease

- Tissue interactions in the joint organ:
  - The synovium is the preferred localization of inflammation. Synovitis and joint effusion contribute to pain and loss of function
  - Both innate and adaptive immune responses may be involved in OA-associated synovitis
  - The hypothesized inverse relationship between OA and osteoporosis is supported by animal model data using Frzb knockout mice

- Osteophyte formation:
  - Osteophytes in large joints and in the hand may be different in nature
  - Osteophytes in large joints can be considered as stabilizing repair efforts

**Research agenda**

- Close interactions between human genetic research and the development of new animal models of OA are required to further advance our understanding of cartilage and joint biology. In particular, complex genetic models including tissue-specific and/or inducible knockout animals need to be developed
- The roles of cortical and subchondral bone, and their impact on the biomechanical properties of the articular cartilage, need to be further defined. From a clinical research point of view, the impact of exercise on bone and cartilage needs to be carefully evaluated. In this context, genotype of patients involved might be important to understand outcomes

**ACKNOWLEDGEMENTS**

Rik Lories is the recipient of a postdoctoral fellowship from the Scientific Research Foundation Flanders (FWO-Vlaanderen). His work is supported by FWO-Vlaanderen and KU Leuven (GOA grant).

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