

Bone Morphogenetic Protein signaling in joint homeostasis and disease

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Abstract

Despite advances in therapies that target inflammation and tissue destruction in chronic arthritis, stimulation of tissue repair and restoration of joint function, the ultimate goal of treatment, is far from achieved. We introduce a new paradigm that may help to improve our understanding and management of chronic arthritis. The presence or absence of tissue responses distinguishes destructive arthritis, steady-state arthritis and remodeling arthritis. Increasing evidence suggests that reactivation of embryonic molecular pathways is an important mechanism to stimulate postnatal tissue repair. Bone Morphogenetic Proteins (BMPs) have critical roles in skeletal development and joint morphogenesis, but also in postnatal joint homeostasis and joint tissue remodeling. Therefore, modulation of BMP signaling may be an attractive therapeutic target in chronic arthritis to restore homeostasis and function of synovial joints.

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1. Introduction

The bones of the skeleton are connected by different types of joints [1]. *Synarthroses* are fibrous zones between the cranial bones. They prevent motion and frequently close at the end of growth. *Amphiarthroses* are typically present in the sacroiliac joints and the spine. They consist of fibrocartilage and allow for limited motion. Most joints in the body are *diarthroses* or synovial joints. They permit a wide range of precise movements. The synovial joint should be considered as a complex organ that is composed of different tissues: the articular cartilage, the underlying subchondral bone, the synovial membrane, the joint capsule, associated tendons and ligaments and eventually menisci (Fig. 1). Their balanced cooperation allows the individual to move. This function requires low-friction contact between the bony bearings, efficient lubrication of the contact surfaces and multidimensional stabilizing forces that prevent dislocation. The continuous strain carried by the synovial joints, requires an enduring homeostatic process in

which the balance between anabolic and catabolic processes is tightly regulated.

Chronic joint diseases are a major health problem. Although these disorders seldom lead to direct mortality, the economic burden caused by progressive morbidity, loss of function and disability remains a challenge to our society [2]. The outcome and severity of diseases such as osteoarthritis (OA), rheumatoid arthritis (RA) and spondyloarthritis (SpA) is determined by the balance in the joint between destructive and homeostatic or reparative pathways (Fig. 2) [3]. Catabolic effectors include pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α), prostaglandins, tissue destructive enzymes such as matrix metalloproteinases (MMP) and cathepsins and cells such as osteoclasts. Systemic and local anabolic tissue responses include anti-inflammatory cytokines such as IL-10, IL-1 receptor antagonist, co-stimulatory regulator molecules such as CTLA4, tissue inhibitors of MMPs (TIMPs) but also growth and differentiation factors.

The final goal of treatment in all chronic diseases is not only the inhibition of excessive tissue destruction but also the restoration of homeostasis and eventually the stimulation of tissue repair. In arthritic disease in particular, modulation

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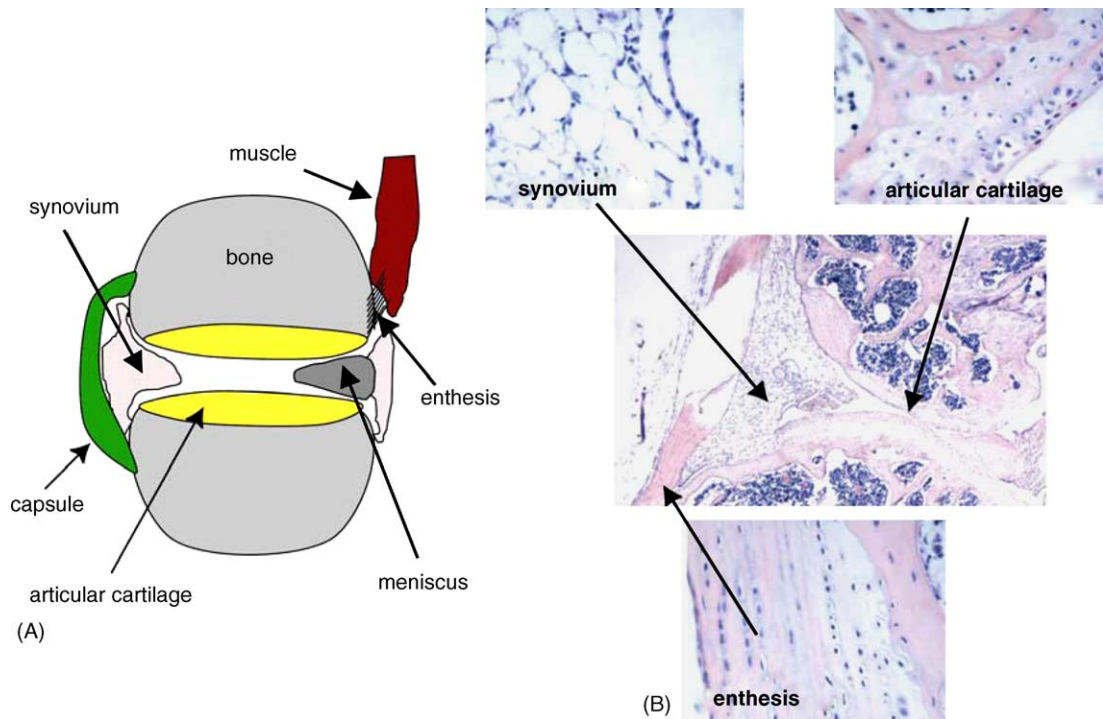


Fig. 1. The anatomy of the synovial joint. (A) Schematic drawing of a synovial joint. The articular surfaces are connected by the synovium that consists of a thin pseudo-epitheloid lining layer and a loose connective tissue sublining zone. In some joints a fibrocartilaginous meniscus is found. The joint is encapsulated. The enthesis is an anatomic region referring to the insertion of tendons and ligaments in the underlying bone. (B) Microscopic image of a knee joint in the mouse. Images of the synovium, the articular cartilage and the enthesis are further enlarged.

of inflammatory and destructive pathways may not be sufficient to achieve restoration of joint function. Increasing evidence suggests that molecular pathways important during development and growth are reactivated in homeostasis and

repair [4–6]. From this point of view, embryonic molecular signaling molecules such as Bone Morphogenetic Proteins (BMPs) become relevant in the context of chronic arthritis not only to understand the complex pathophysiology of

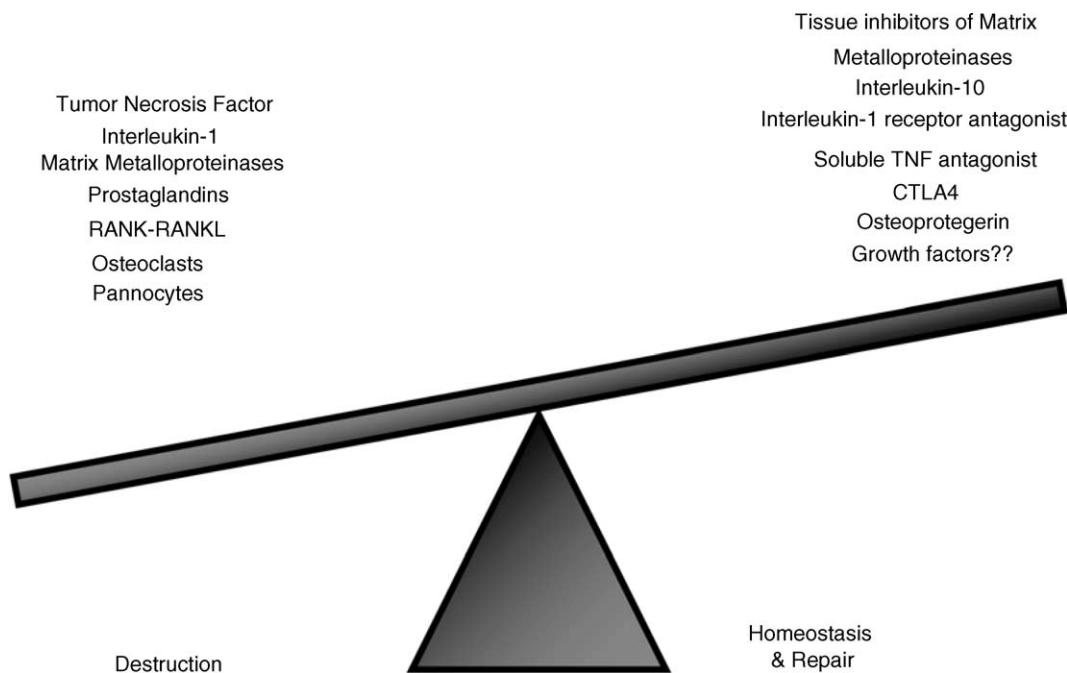


Fig. 2. The outcome and severity of chronic arthritis is determined by the balance between destructive or pro-inflammatory and homeostatic or regenerative signaling pathways.

disease but also to develop regenerative therapeutic strategies.

The Bone Morphogenetic Proteins and closely related Growth and Differentiation Factors (GDFs) are a large group of structurally related polypeptides that belong to the transforming growth factor- β (TGF- β) superfamily [7,8]. Within the BMP/GDF family, different groups can be further distinguished based on structural homology (Table 1) [7–9]. The original discovery of BMPs as protein factors that induce both ectopically and in vivo a cascade of endochondral bone formation [10–13], has strongly stimulated the study of their function in skeletal development (for review, see [14,15]) and joint morphogenesis [16–20]. However, it has become increasingly clear that BMPs are involved in a wide array of biological processes both during development and in postnatal life [21,22]. These processes include the specification of the dorso-ventral body axis and the development, growth and homeostasis of most organs. BMPs can act as morphogens, growth factors or cytokines depending on their spatio-temporal expression and target cells. Their downstream effects include cell lineage determination, differentiation, motility, adhesion and death [22].

In their canonical pathway, homodimeric BMPs induce ligand-dependent type I and II receptor heterodimerization. These receptors are transmembrane serine–threonine kinases and phosphorylate intracellular receptor-smad signaling molecules (R-smad1/5) that bind common-smad4 (co-smad4) and then translocate to the nucleus [7,9,23] (Fig. 3). The diversity of cell responses to BMPs can at least partially be explained by differences in the affinities of distinct ligands for specific type I and II receptor combinations [7,9,24]. For instance, the BMP-receptor type Ia (BMP-RIa, also known as Activin-like kinase-3 (Alk-3)) can bind both BMP2/4 and BMP 6/7 in combination with either the BMP type II receptor, Activin type IIa receptor or Activin type IIb receptor [25–27]. BMP signaling is further regulated by extracellular antagonists such as noggin, chordin, gremlin, the DAN/Cerberus family, follistatin, follistatin-related protein and sclerostin (for review see [28]), by accessory receptors such as endoglin [29] and BAMBI [30] and by intracellular inhibitors such as inhibitory-smads (I-smad6/7) [31,32]. Transcriptional responses to BMP signaling are tightly controlled by different co-activators and co-repressors (for review see [7,24]). In addition, ligand and receptor availability is dependent on propeptide processing [33] and endocytic trafficking, respectively [34].

In this review, we introduce a new paradigm of arthritic disease with the focus on the balance of damage and repair. We further discuss and position the potential relevance of BMPs in this paradigm.

2. Joint homeostasis

The function of the synovial joint is to connect the elements of the skeleton and to allow motion. To achieve

these goals, the joint is a well-organized organ in which different tissues interact to provide an agile structure with low-friction contact, lubrication of different surfaces and external and internal stabilization. A thin layer of *articular cartilage* is found at the edges of the skeletal bones. It belongs to the postnatal cartilaginous skeleton and is considered “stable”, in contrast to the transient cartilage tissue. The unique biological properties of the articular cartilage include resistance to vascular invasion, mineralization and replacement by bone. The developmental origin of the articular cartilage has been studied extensively (for review see [20]). A unique precursor cell population expressing GDF5/Cartilage Derived Morphogenetic Protein-1 (CDMP-1) in the joint interzone during joint morphogenesis has been identified [16–19,35,36]. The articular cartilage is composed of cells, the articular chondrocytes, and their extra-cellular matrix, composed of collagens, mainly type II fibrils and proteoglycans. These macro-molecules are glycosylated proteins in which the glycoconjugates are formed by the addition of sulfated glycosaminoglycan side chains on a core protein. Aggrecan is the most important proteoglycan in articular cartilage. Other proteoglycans of the articular cartilage include decorin, biglycan and fibromodulin. The triple helical type II collagen fibers arrange with minor collagen components (types VI, IX, XI, XII and XIV) to form a fibrous network that entraps the proteoglycans and conveys tensile strength to the tissue. The very condensed and abundant negative charges on the proteoglycans attract and expulse water during compression and decompression. The tissue is critically dependent on diffusion of solutes from the joint fluid or the subchondral bone. Articular chondrocytes can react to very diverse stimuli that influence their anabolic and catabolic activity. They have a limited repair capacity and fail to repair damaged cartilage. The underlying *subchondral bone* has very dense trabeculae in a characteristic architecture that is important for stress distribution. The precise biological role of the subchondral bone is still unclear but a tight molecular interaction with the articular cartilage is very likely [37].

The *synovial membrane* connects the bones and lines the inner cavity of the joint together with the articular cartilage. Under physiological circumstances, this membrane has a thin lining layer usually 1–3 cell layers thick. The sublining zone is a loose and vascular connective tissue with few cells. Two different cell types are present in the lining layer: type A synoviocytes or macrophages and type B synoviocytes or fibroblast-like cells. This latter cell population is at least partially derived from the joint interzone cells. However, many aspects of the biology of the pseudo-epithelial type B synoviocytes remain unclear. These cells show some remarkable properties. They are capable of anchorage-independent growth in vitro [38] and contain a population of mesenchymal stem cells [39]. The normal synovium is collapsed upon itself and onto the articular cartilage. The joint cavity contains a minimal amount of synovial fluid necessary for boundary-layer lubrication [1]. The synovio-

Table 1
Bone Morphogenetic Proteins and the Transforming Growth Factor super-family

Subgroup	Name	Alternative names
BMP2/4	BMP2	BMP2A
	BMP4	BMP2B
BMP5/6/7	BMP5	Vg1-related sequence, Vgr1 Osteogenic protein-1, OP1 Osteogenic protein-2, OP2 Osteogenic protein-3, OP3
	BMP6	
	BMP7	
	BMP8A BMP8B	
GDF1	GDF1	Vgr2
	GDF3	
GDF5/6/7	GDF5	Cartilage Derived Morphogenetic Protein-1, CDMP-1
	GDF6	CDMP-2, BMP13
	GDF7	BMP12
BMP3	BMP3	Osteogenin
	BMP3b	GDF10, Sumitomo-BIP
BMP9/10	BMP9	GDF2
	BMP10	
GDF9	GDF9	BMP15 BMP-placenta, PLAB, prostate-derived factor, PDF, macrophage inhibiting cytokine-1, MIC1
	GDF9b	
	GDF15	
GDF8	GDF8	Myostatin BMP11
	GDF11	
TGF- β	TGF- β 1	
	TGF- β 2	
	TGF- β 3	
Activins	Activin A	
	Activin B	
	Activin AB	
Inhibins	Inhibin A	
	Inhibin B	
Nodal	Nodal	
Lefty	Lefty1	LeftyA, endometrial bleeding associated factor, EBAF
	Lefty2	LeftyB
MIS	MIS	Mullerian inhibiting substance

cytes synthesize two important components of the fluid. Lubricin is a small glycoprotein that binds to articular cartilage and can retain a protective layer of water molecules. This molecule is essential for the extreme low-friction state between both cartilages. Hyaluronic acid cushions the synovium – cartilage contact and prevents pinching of the synovial membrane. The thin layer of synovial fluid is resistant to distractive forces and thereby contributes to joint stability.

The *joint capsule*, *ligaments* and *tendons* are strong and organized connective tissues that have critical stabilizing functions. Ligaments restrict motion into undesired axes. Muscles and tendons provide further constraints in the

desired axes of motion. The insertion of tendons, ligaments and capsule into the underlying bone, is an anatomical zone called the *enthesis* [40]. This zone consists of four different layers: fibrous tissue, uncalcified fibrocartilage, calcified fibrocartilage and bone. The different layers are thought to provide a gradual change in mechanical properties at the insertion site, so that stress concentration is dissipated [40]. This zone is of particular importance in SpA and degenerative joint disease.

3. A new paradigm of joint diseases

Chronic joint diseases can be conceptualized from different angles. The clinician needs to diagnose, classify and treat specific disorders. The scientist wants to understand the mechanisms of disease looking at the target tissues, genetic and environmental factors, critical molecular signaling and specific features of the disease processes. It is the latter approach that should ultimately define our therapeutic strategies.

Three major forms of chronic joint disease are classified in clinical practice. OA is by far the most common form [41]. It is often considered degenerative or “tear and wear” joint disease since its prevalence increases with age and the disease process is associated with environmental risk factors such as high body mass index and preceding trauma. OA is not a normal phenomenon of aging and involves dynamic biochemical, biomechanical and cellular processes affecting the whole joint organ. For instance, inflammatory reactions contribute to joint pain, swelling and loss of function. The clinical spectrum varies from disease in a single joint (monoarthritis) to a poly-articular disorder [41,42]. Current treatment strategies primarily aim to control pain. No specific treatments are available that alter the pathological course of the disease process itself. For severe forms with loss of joint function, joint replacement surgery is the only solution.

RA is a chronic systemic inflammatory disease that primarily affects the synovial joints. A symmetrical polyarthritis in the diarthrodial joints of the appendicular skeleton, in particular of hands and feet is characteristic [43]. Involvement of the axial skeleton is rare with the exception of the odontoid process of the second cervical vertebra (C2) and the transverse ligament of C1, leading to instability of the odontoid articulation between C1 and C2 and potentially to cervical myelopathy. Extra-articular manifestations are regularly encountered and include subcutaneous nodules, vasculitis, pleuritis, pericarditis, anemia of chronic disease, neurological signs such as mononeuritis multiplex, secondary Sjögren’s syndrome and Felty’s syndrome [43]. The disease affects more women than men (2.5:1 female:male ratio) and typically develops between the third and sixth decade. Classical treatment strategies have employed anti-inflammatory and immune suppressive treatments with moderate success. The recent introduction of so-called biological response modifiers such as TNF-blockade through

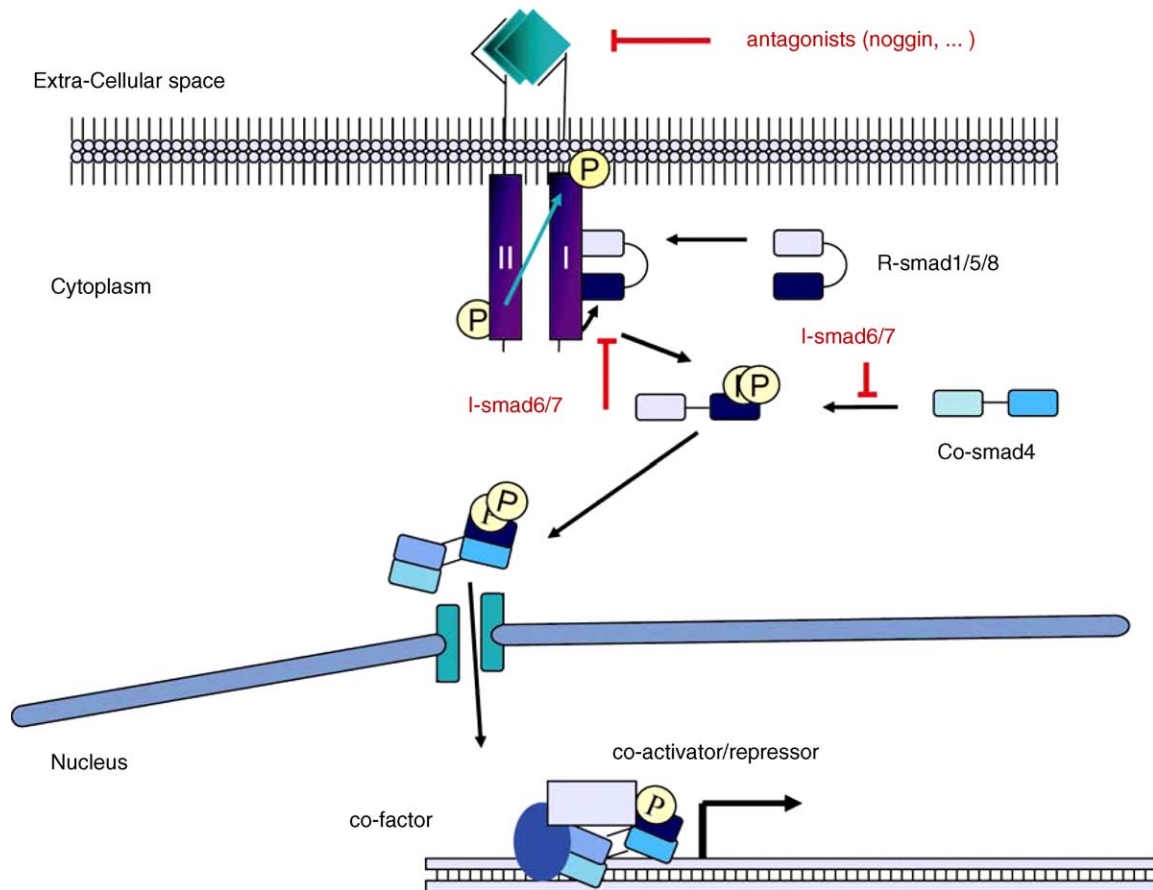


Fig. 3. The canonical BMP molecular signaling pathway. Ligand dimers are binding type II and type I receptors. The type II receptor serine–threonine kinase phosphorylates the type I receptor. The type I receptor further phosphorylates a receptor-smad (R-smad). The R-smad forms a complex with common-smad4 that translocates to the nucleus. Associations of the smad complex with co-factors and/or co-activators or repressors result in regulation of gene transcription. Soluble antagonists and inhibitory-smads (I-smad) add to the complexity of this signaling pathway.

monoclonal antibodies or soluble receptors has greatly improved the success of therapy [44,45] and suggest that in the near future control of inflammation may be achieved in a majority of patients.

The SpAs are a group of chronic inflammatory joint disorders with a prevalence of about 0.4%, primarily affecting young individuals [46]. Different diagnostic entities, based on clinical, genetic and pathological characteristics, are classified within this disease concept. These include ankylosing spondylitis, psoriatic arthritis, reactive arthritis, arthritis associated with inflammatory bowel disease, a juvenile and an undifferentiated form. Axial involvement, in particular of the sacroiliac joints is a common feature. Peripheral arthritis is often oligoarticular and asymmetric. However, forms of SpA without axial involvement and characterized by symmetrical polyarthritis do occur. Enthesitis, e.g. Achilles tendon inflammation and fasciitis plantaris, is a typical sign of SpA. Uveitis, psoriasis and inflammatory bowel disease are frequent systemic manifestations. Gender distribution is skewed toward male predominance in ankylosing spondylitis, reactive arthritis and juvenile SpA (sex ratio female:male 1:3) but is less characteristic in psoriatic arthritis and SpA associated with

inflammatory bowel disease [47]. The disease process often starts in early adulthood. However late onset SpA, in particular psoriatic arthritis is not uncommon. Immunosuppressive and anti-inflammatory drugs have a limited effect on symptoms and disease progression. Like in RA, the recent introduction of anti-TNF drugs has a profound impact on patient care [48,49] but the long-term benefits of these strategies remain to be proven.

Some differences between these distinct types of arthritis can partially be understood by differences in the primary target tissue of the disease process within the joint (Fig. 4). OA is most probably a disease of the cartilage, also closely involving the subchondral bone, with secondary changes in the whole joint organ. Early events in the cartilage include fibrillation and microscopic ulceration. As the disease progresses, the cartilage gradually disappears and the subchondral bone undergoes alterations including osteophytosis, sclerosis and cyst formation. Pro-inflammatory cytokines such as IL-1 and TNF- α and enzymes such as MMPs play a central role in the degradation of cartilage. RA is generally thought to be a disease of the synovium. At the microscopic level, the disease process is characterized by hyperplasia of the lining layer and inflammatory cell

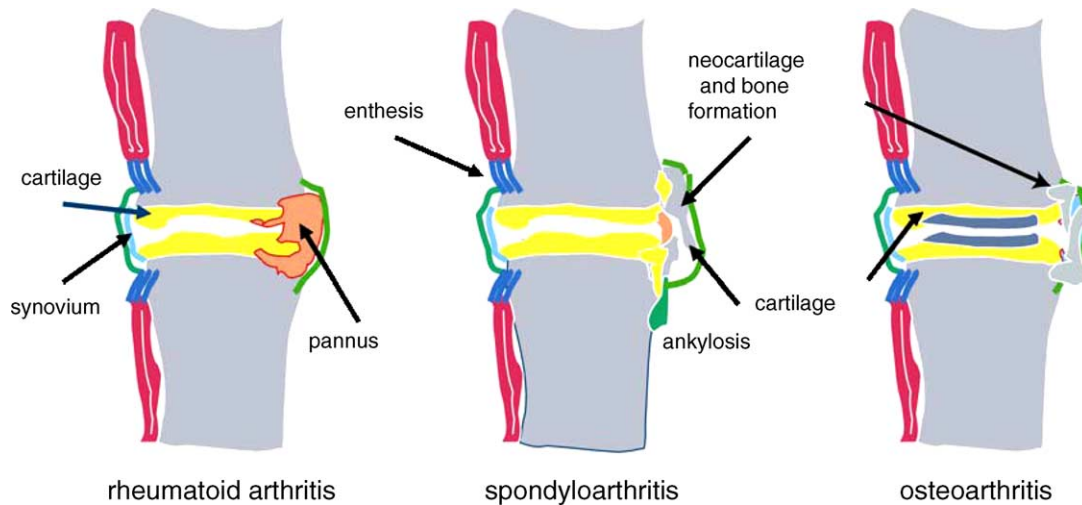


Fig. 4. Differences in the primary target tissue in the joint organ are seen in distinct forms of chronic arthritis. In rheumatoid arthritis the synovium is the likely target tissue. Parts of the inflamed synovium transforms into pannus, an aggressive tissue mass that erodes cartilage and bone. Little or no repair is recognized. In spondyloarthritis, the enthesis is primarily involved. Inflammation at the insertions is leading to local new cartilage and bone formation that may progressively lead to joint ankylosis and loss of joint function. In osteoarthritis the cartilage and subchondral bone are first affected. Progressive loss of cartilage results in remodeling of the whole joint with synovial inflammation and local cartilage and bone formation (osteophytes).

infiltration in the sublining zone. Parts of the synovium transform into so-called *pannus* tissue, a proliferating cell mass that also contains osteoclasts and that erodes cartilage and bone [50,51]. The radiological appearance of the disease is therefore characterized by erosions at the joint margins. Increasing evidence suggests that in SpA the primary target of the pathological process is the enthesis [40,52]. Ankylosing enthesitis is a local inflammatory reaction followed by heterotopic cartilage and bone formation, eventually leading to joint space bridging. It is considered the hallmark of SpA [40,52]. Synovitis is also a feature of many patients with SpA. Erosive joint destruction often appears less outspoken than in RA but joint ankylosis is a major cause of morbidity and disability.

The response to injury provides a new and exciting angle to think about chronic arthritis. Loss of function of synovial joints is the result of a destructive process with insufficient repair associated with an imbalance in pro- and anti-inflammatory cytokines [3] but also between tissue destructive and homeostatic/reparative molecular signaling pathways. Our understanding of the critical cytokines involved in the chronic inflammatory processes has evolved into targeted therapies such as TNF blockade in RA and SpA. While this therapy is successful at arresting the disease progression, it does not appear to promote a reparative response of the damaged tissue. Disease remission may only be achieved by adequate blocking of inflammation together with appropriate activation of local tissue repair processes, ultimately leading to restoration of joint homeostasis and its proper function. This concept has important consequences not only for our understanding of the disease processes but also for the development of new therapeutic targets specifically aimed at restoring joint homeostasis and repair, the goal of regenerative medicine.

In RA, considered as an auto-immune disease, very little repair, if any, is observed, even with control of the inflammatory process. In OA, a disease with limited inflammation, local repair responses may be sufficient for many years, but ultimately joint homeostasis is lost and results in joint failure. In SpA, inflammation and excessive reparative responses may lead to inappropriate cartilage and bone formation causing joint ankylosis that thereby contributes to loss of joint function. However, in this concept of arthritis, existing clinical disease boundaries are of lesser significance. Regenerative medicine approaches may benefit from defining the pathological process as a “destructive” arthritis, typical for RA, a “steady-state” arthritis, as seen in OA and a “remodeling” arthritis, more characteristic for SpA. Some forms of psoriatic arthritis and rapidly progressive OA may rather fit in the destructive arthritis group whereas non-erosive rheumatoid factor negative patients with mild RA may belong to the steady-state arthritis group. Joint erosion in RA or SpA may lead to secondary OA even after complete control of the inflammatory process has been achieved but without restoration of the joint homeostasis.

Since many aspects of tissue repair mimic embryonic tissue formation [4–6], it is anticipated that signaling pathways critically involved in the formation of joints such as Bone Morphogenetic Proteins, may provide a complementary or alternative therapeutic approach in patients with chronic arthritis.

4. BMPs and articular cartilage homeostasis and repair

The homeostasis of articular cartilage is critically dependent on the balance between anabolic and catabolic

molecular pathways. Articular chondrocytes have a limited repair capacity after trauma [53]. Chondral lesions without injury to the subchondral bone do not heal spontaneously and gradually worsen. Osteochondral defects penetrate into the bone and show some attempts of repair with invasion of mesenchymal progenitor cells from the subchondral bone marrow cavities. However, no articular cartilage but fibrocartilage appears to be formed. Nevertheless, the complex regulation of extracellular matrix synthesis suggests that the articular chondrocytes can retain cartilage homeostasis to a certain degree or for a limited period in case of chronic or progressive strain such as seen in osteoarthritis.

Different factors may lead to net extracellular matrix loss and progressive cartilage degradation [41]. These include inflammation and activation of tissue destructive enzymes such as MMPs, age-dependent decline in extracellular matrix synthesis capacities with or without decreased responsiveness to growth factors, excessive strain or progression of minor cartilage injuries. To evaluate the importance of BMPs/GDFs in the homeostasis and repair of articular cartilage distinct but complementary approaches have been taken: identification of BMPs and activation of their signaling pathway in arthritic joints, *ex vivo* and *in vivo* studies, and genetic models in mice and men. Importantly, recent *in vivo* data obtained in animal models seem to corroborate the findings that have been described in *in vitro*, *ex vivo* and genetic studies.

Ex vivo data convincingly demonstrated the presence of different BMPs in normal and osteoarthritic cartilage. Most of these studies were performed on human tissue. The CDMPs and BMP7 were studied quite extensively. CDMP-1 and CDMP-2 were present in normal human adult articular cartilage [54]. Its expression, as determined by immunohistochemistry, was mostly restricted to the superficial layer of cartilage in normal joints, while in osteoarthritic cartilage the expression domain further extended into damaged areas [54]. BMP7 was demonstrated in normal adult and osteoarthritic human articular cartilage by *in situ* hybridization, Western blotting and immunohistochemistry [55]. BMP7 mRNA was found in the superficial and middle layers of the cartilage, whereas in the deep layer levels of expression were very low. The topographic distribution of the protein within the tissue was interesting as revealed by immunostaining performed with two different antibodies, one that recognizes the active mature form, and the other that reacts with the inactive pro-form [55]. Mature BMP7/OP-1 was found predominantly in the superficial and middle layers of the tissue, whereas pro-BMP7/OP-1 was predominantly detected in the deep layer of the cartilage. The distinct localization of pro- and mature forms of BMP7/OP-1 suggests that the processing of pro-BMP7/OP-1 into mature BMP7/OP-1 may occur primarily in the superficial chondrocytes. In further studies levels of BMP7 protein and mRNA were reduced with increasing age or progression of osteoarthritis [56]. It is not clear however whether this is a primary or a secondary event. It is noteworthy that the levels

of BMP7 mRNA are fairly low. Bobacz et al. [57] failed to demonstrate BMP7 mRNA in osteoarthritic cartilage with conventional PCR methods that are less sensitive than the procedures used in the studies described above [55,56].

Downstream mediators in the canonical BMP signaling pathways (R-smads 1/5 and co-smad 4) were demonstrated in normal and osteoarthritic cartilage by PCR and immunohistochemistry [58]. Expression of these smads was upregulated *in vitro* in chondrocyte cultures on alginate beads. None of the smads however was up or down regulated in osteoarthritic cartilage as compared to normal controls [58]. Inhibitory-smads6 and 7 were also demonstrated in normal and osteoarthritic human cartilage [59]. IL-1 augmented the expression of smad7, but downregulated the expression of smad6 *in vitro* [59].

BMP antagonists such as chordin, follistatin and gremlin but not noggin were found in articular chondrocyte cultures [60]. Expression of follistatin and gremlin was upregulated in osteoarthritic chondrocytes as compared to normal controls. TNF- α and interferon- γ significantly stimulated follistatin expression but down-regulated the expression of gremlin. IL-1 had no effect on follistatin but reduced gremlin expression. Conversely, BMP2 and BMP4 significantly stimulated expression of gremlin but down-regulated that of follistatin. Immunohistochemistry revealed localization of follistatin and gremlin in the superficial layers of the cartilage. Another BMP antagonist, chordin-like protein-2 was also identified in articular cartilage [61]. In normal samples, only a few chondrocytes in the superficial and middle zone expressed chordin-like protein 2 mRNA. In contrast, in a large number of OA samples, expression was enhanced in the middle zone, in particular in clustered cells, but absent in the superficial layer. Remarkably in 2 RA cartilage samples, weak expression was seen in both superficial and middle zones. Taken together, these data suggest that delicate balances are regulating the net effect of BMP signaling in a very dynamic process.

The specific roles of BMPs in articular cartilage and the factors that regulate their expression or processing are more difficult to understand. There is ample *in vitro* evidence that different BMPs are capable of stimulating proteoglycan and collagen type II synthesis in different systems both in the presence and absence of serum. Different studies demonstrated an anabolic role for BMP3 [62], BMP4 [63,64], BMP7 [65–70] and CDMP-1 [54,57] in proteoglycan synthesis. Remarkably, the sensitivity of articular chondrocytes to BMP signaling *in vitro* is dependent on the age of the donor [63,64]. As OA is predominantly a disease of older age, this may have important consequences on our understanding of the balance between anabolic and catabolic molecular signaling in the progression of this disease. As BMP7 is associated with differentiation of chondrocytes towards hypertrophy during development, it is noteworthy that this effect is not seen in *in vitro* cultures of articular chondrocytes [65,68–70]. Recombinant CDMP-1 increases proteoglycan biosynthetic activity in adult articular cartilage

that has been partially matrix-depleted by mild trypsin treatment, a model that mimics some aspects of osteoarthritis [54].

Increasing evidence suggests interactions between BMP signaling and cytokine networks [58–60,71–74]. IL-1 is considered one of the main pro-inflammatory cytokines that plays a role in cartilage destruction. Upregulation of BMP2 has been demonstrated in cultured articular chondrocytes [72]. Effects on BMP7 have been studied more in detail showing dose-dependent differences in the cytokine effect [74]. Low dose of IL-1 resulted in enhanced BMP7 mRNA expression and protein synthesis. However, at higher doses, the effect on transcription was no longer accompanied by an effect on protein translation.

Intra-articular injections of BMP2 in the mouse knee have also been used to assess the effect of this BMP on articular cartilage in vivo [75]. BMP2 stimulates proteoglycan synthesis in normal knees but cannot do this in a model of destructive arthritis [76]. Similarly BMP2, in contrast to TGF- β , was not capable of counterbalancing the effect of IL-1 on articular cartilage after intra-articular injection [75]. The use of BMP technology in tissue engineering approaches is beyond the scope of this review. It is clear however that the complexities of BMP signaling such as processing and ligand–antagonist and ligand–receptor interactions, require further advances in basic biology knowledge and careful design of the procedures, carriers and molecules in these type of experiments.

Null mutations in different BMPs and their receptors are either early embryologically lethal [77–79] or have severe defects in joint patterning and morphogenesis [16,35,80] that interfere with analysis of acquired joint disease in postnatal life. To overcome this problem, Rountree et al. [36] developed a conditional gene deletion system that takes advantage of the expression in the joint interzone during morphogenesis of GDF5, the murine homolog of CDMP-1 [81]. Heterozygous *BMPRIa*^{+/-} mice, engineered to express a Cre recombinase in the *Gdf5* locus (*GDF5*^{Cre/Cre}; *BMPRIa*^{+/-}) were crossed with mice that carry a floxed *BMPRIa* allele (*GDF5*^{+/+}; *BMPRIa*^{floxP/floxP}). The *GDF5*^{+Cre}; *BMPRIa*^{-floxP} conditional knockout progeny were viable and showed some developmental defects (short ears, soft tissue syndactyly between digits 1 and 2 and tarsal joint ankylosis). Importantly, *GDF5*^{+Cre}; *BMPRIa*^{-floxP} mice failed to maintain postnatally articular cartilage in many joints as compared to litter mate “control” (*GDF5*^{+/-}; *BMPRIa*^{+floxP}) mice. At birth the digit joints appeared normal with high expression of both aggrecan and collagen type II mRNA in the two groups. As soon as 1 week after birth and more clearly by 2 weeks, changes in the articular cartilage had occurred. Expression of proteoglycans and collagen type II was greatly reduced. In other joints of forefeet and hindfeet similar changes were observed at 7 weeks. By 9 months of age many regions of the cartilage were severely damaged. Progressive changes were also observed in the knee joints and induced a loss of function.

Surprisingly, in the ankle region, cartilage loss was accompanied by synovial hypertrophy and even some invasion of this tissue into the underlying bone. The fact that this observation is unique for the ankles suggests a secondary phenomenon caused by joint instability as a consequence of the rapid loss of articular cartilage. However, a primary effect due to defective BMP or GDF5 signaling cannot be excluded. Further more circumstantial evidence has come from genetic studies in humans. Polymorphisms in the *BMP2* gene have been associated with osteoarthritis [82]. In summary, these data provide strong in vivo evidence for the critical function of BMP signaling in the homeostasis of articular cartilage and the joint.

5. Bone Morphogenetic Proteins and the synovium

The development of needle arthroscopy as a diagnostic tool in daily rheumatology practice, and the availability of biopsies at distinct stages of the disease, is rapidly increasing our knowledge of the pathology of arthritis and the molecular players involved. We have demonstrated that different BMPs, including BMP2, BMP6 and BMP7 are expressed in synovial biopsies obtained from patients with chronic arthritis [73]. Protein levels of BMP2 and BMP6 are significantly higher in patients with RA and SpA as compared to non-inflammatory controls. BMP2 and BMP6 protein is found in both macrophages and fibroblast-like synoviocytes as demonstrated by immunohistochemistry [73]. BMP2 and BMP6 expression in fibroblast-like synoviocytes in vitro is upregulated by pro-inflammatory cytokines such as IL-1 and TNF- α , but not by interferon- γ . We also demonstrated that BMP2 is associated with fibroblast-like synoviocyte apoptosis in vitro and in vivo [73]. These data give additional support to the hypothesis that BMPs are pleiotropic cytokines with different function in the complex signaling network in chronic arthritis. As described above, upregulation of BMP2 expression by IL-1 has also been demonstrated in articular chondrocytes in vitro [72].

Clinical findings indicate that the presence of BMPs in the synovium is apparently not associated with bone formation in this tissue. This suggests that the autocrine or paracrine effects of BMPs in the joint may be of a different nature than the predicted ectopic effects of BMPs in soft tissues [10,83]. We have detected different BMP antagonists in synovial tissues by RT-PCR including follistatin, gremlin and chordin (unpublished observations). Follistatin and gremlin were also identified by RT-PCR in fibroblast-like synoviocyte cultures [60]. This suggests that the role of BMPs in synovial tissues is determined by local balances with antagonists and is likely also to be dependent on processing of BMPs by enzymes such as furins. Identification of BMP target cells in synovial tissues in both normal and arthritic samples will provide additional

insights in the role of BMPs in the synovium. At present, little is known about potential target cells. Expression of different BMP receptors is present in fibroblast-like synoviocyte cultures [39].

As BMP signaling in development typically affects progenitor cell populations in the mesenchymal lineage, we would expect the mesenchymal cells in postnatal tissues to be a target for BMP signaling. Interestingly, BMP-RIa positive cells have been identified as potential mesenchymal stem cells in both RA [84] and joints from mice with collagen-induced arthritis, a model of RA [85]. Surprisingly, infiltration into the synovium from the bone marrow is apparently preceding the onset of symptoms in the induced model and a specific role for this cell population in disease pathogenesis is hypothesized by the authors [85]. Fibroblast-like synoviocytes express all BMP receptors and many BMPs, as determined by RT-PCR [39]. The immunohistochemical studies did not detect BMP receptors of any type in normal synovial membrane [84]. This apparent discrepancy may have different explanations. The cell isolation technique and the subsequent expansion of this selected cell subpopulation can enrich in BMP receptor expressing cells. In addition, cells in culture are exposed to an environment that is different from the native tissue, and therefore change their molecular phenotype. It is also not excluded that the antibodies used for immunohistochemistry are not able to detect all BMPRIa receptor positive cells in the synovial membrane with sufficient sensitivity.

Increasing evidence supports the hypothesis that multipotent stem cells are available postnatally in different organs and tissues. These cells could contribute to postnatal growth, and participate in tissue homeostasis by replacing differentiated cells lost to physiological turnover, injury and senescence [86]. A hypothetical role for BMPs in adult tissues can be the maintenance, recruitment and differentiation of a pool of progenitor cells for tissue homeostasis and regeneration. Fine balances of BMPs would be required for either the maintenance of this cell population in a quiescent phenotype, or their activation and commitment to a specific lineage. Further studies are required to see whether BMPs in the synovium have a direct effect on inflammatory processes and the organization of the synovial architecture in homeostasis and disease.

6. BMPs and joint remodeling

Joint remodeling can be considered as a consequence of strain or damage. Anabolic efforts to preserve joint integrity may often appear unnoticed as they stay within the anatomical borders of the joint. However, in specific situations, metaplastic changes are occurring and new tissue formation becomes apparent. In OA, this process of osteophyte formation at the joint margins is often considered a defensive mechanism to increase joint stability. It seldom contributes to the symptoms. In SpA in contrast, the

formation of enthesophytes leading to joint ankylosis contributes directly to the pathology and morbidity of the disease. From this point of view, it becomes clear that reactivation of embryonic signaling pathways in tissue responses may be a defensive mechanism but that inappropriate or untimely activation of these pathways may be a critical factor in the disease process itself.

The role of BMP and TGF- β signaling in osteophyte formation in OA has been extensively studied [75,87–89]. Immunohistochemical studies in humans indicate that each BMP has a distinct pattern of distribution [87]. Immunoreactivity for BMP2 was observed in fibrous tissue as well as in osteoblasts. BMP3 was mainly present in osteoblasts. BMP6 was restricted to young osteocytes and bone matrix. BMP7 was observed in hypertrophic chondrocytes, osteoblasts and young osteocytes of both endochondral and intramembranous bone formation sites. CDMP-1, -2 and -3 were strongly expressed in all cartilage cells.

Injection of recombinant BMP2 in normal knees results in increased proteoglycan synthesis but also in neo-cartilage and bone formation [75,88]. In contrast to what is seen after the injection of TGF- β , the osteophytes are not localized at the margin between cartilage and bone but rather at the growth plate [88,90]. Also in contrast with TGF- β , injection of BMP2 does not counterbalance the catabolic effect of simultaneous IL-1 injections [88]. BMP expression was upregulated in synovium and cartilage after papain-induced osteoarthritis [91]. Overexpression of inhibitory-smads is successful at preventing osteophyte formation in this system [91]. In further studies, these authors could demonstrate that synovial macrophages are critical in this model [89]. Together with our results obtained in humans [73], these data provide the first evidence that BMPs may interact with cells of the immune system in the synovium. Such interactions are likely to occur and further influence the immune response as has also been demonstrated in inflammatory bowel disease [71].

It is noteworthy that in TNF transgenic mice that develop a severe destructive arthritis, new bone formation in the subchondral bone is stimulated by BMPs originating from B cells in the bone marrow [92]. The intracellular signaling pathway that is activated in specific cell types or under specific circumstances may critically influence the outcome of BMP signaling in cell metaplasia. In this respect, it was recently described that activation of the smad pathway in fibroblast-like synoviocytes leads to chondrogenic differentiation and inhibits terminal hypertrophy of the chondrocyte. In contrast activation of p38 signaling promotes terminal differentiation [93]. Again, these results support a role for BMP signaling in repair and homeostasis.

7. Conclusion and future directions

Stimulation of tissue repair and restoration of joint homeostasis and joint function is the ultimate goal of therapy

in chronic arthritis. Endogenous anabolic molecular signaling pathways are activated in response to stress or injury. Depending on the nature and strength of the destructive forces, these endogenous mechanisms are to some extent capable of resisting the structural damage to the joint organ. In most cases however the balance is ultimately lost and progressive joint destruction and loss of function occurs. In remodeling arthritis, repair mechanisms are apparently ill coordinated or wrongfully activated. In SpA in particular this may contribute directly to pathology and loss of joint function. The BMP pathway is likely to be involved in many of these processes. The complexity of BMP signaling demonstrates that fine-tuning of this signaling pathway is likely to be a challenge in the development of regenerative therapeutic strategies.

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