Novel targets in bone and cartilage

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The spectrum of arthritis ranges from erosive (e.g., rheumatoid arthritis) to ossifying disease with formation of new bone (e.g., ankylosing spondylitis and osteoarthritis). The molecular basis for these different patterns of arthritis had long been unclear. In the last few years, however, characterisation of catabolic and anabolic molecular pathways in different forms of arthritis led to a better understanding of joint remodelling and revealed novel therapeutic targets.

Recent findings show that catabolic and anabolic molecular pathways govern bone and cartilage remodelling in healthy and arthritic joints. The predominance of catabolic molecular pathways (e.g., receptor activator of nuclear factor-xB ligand (RANKL)/RANK and cathepsin K) causes erosive disease whereas anabolic signalling (e.g., Wnt and fibroblast growth factor (FGF)18) favours the formation of new bone including bony spurs and subchondral sclerosis. Other pathways may have a dual function in arthritis (e.g., hedgehog) leading to either catabolic or anabolic joint remodelling dependent on other factors. Key mediators within these signalling pathways may serve as novel targets for treating pathological remodelling of bone and cartilage in arthritis.

Molecular pathways govern remodelling processes of bone and cartilage in arthritic joints. Future therapies will likely target the pathologic activity of these molecular pathways to specifically block either catabolic or anabolic joint remodelling in arthritis.

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bone (i.e., anabolic, osteophyte-like pattern). Whereas rheumatoid arthritis (RA) displays a catabolic phenotype with cartilage degradation and bone erosions, the anabolic patterns of joint remodelling in ankylosing spondylitis (AS) and osteoarthritis (OA) are marked by a spectrum of new-bone formation, ranging from subchondral sclerosis to bony spurs and joint fusion.

In healthy joints, degradation and formation of bone and cartilage are tightly controlled, which results in a steady state of bone and cartilage tissue. During arthritis, however, the articular homeostasis is severely disturbed; the balance between resorption and formation of bone and cartilage may shift to either of both sides, leading to catabolic or anabolic joint remodelling [1]. In articular bone, osteoclasts and osteoblasts are the key cellular effectors of catabolic and anabolic processes [2,3], whereas chondrocytes preserve homeostasis in cartilage [4].

In arthritis with catabolic remodelling (e.g., RA) osteoclasts degrade bone and cause erosions. Osteoclasts originate from monocytes, which are attracted to the arthritic joint by inflammatory mediators. The differentiation of monocytes into osteoclasts necessitates the presence of receptor activator of nuclear factor-κB ligand (RANKL) among other factors. Of note, inflammatory cytokines such as tumour necrosis factor α (TNFα), interleukin (IL)-1, IL-6 and IL-17 [5–8] can induce RANKL expression and osteoclast formation [9–11]. Finally, bone degradation encompasses two major steps: osteoclasts secrete hydrochloric acid to demineralise bone and release matrix-degrading enzymes, including cathepsins, to digest the bone matrix [12]. In anabolic forms of arthritis (e.g., OA and AS), osteoblasts, rather than osteoclasts, mediate remodelling processes. Osteoblasts derive from mesenchymal progenitors upon stimulation by parathyroid hormone, bone morphogenic proteins (BMPs) and Wnt proteins. Osteoblasts secrete bone-matrix proteins and promote mineralisation, with both processes leading to the formation of new bone.

Whereas mechanical stress is a major determinant of cartilage degradation in OA, pathological activation of catabolic cellular and molecular processes leads to the loss of cartilage in inflammatory types of arthritis. In RA, synovial fibroblasts and neutrophils within the synovial fluid, as well as chondrocytes, can release proteases that degrade cartilage. Interestingly, chondrocytes change their range of tasks from matrix production to release of matrix-degrading enzymes [13].

In the following, we explain the molecular basis for catabolic and anabolic joint remodelling in arthritis. We highlight interesting targets to re-establish tissue homeostasis of bone and cartilage in arthritic joints (Fig. 1). Since some forms of arthritis show generalised involvement of the skeleton (i.e., osteoporosis) beyond local joint remodelling, we will finally address systemic effects of these novel therapeutic approaches.

**RANKL**

RANKL stimulates the differentiation and activation of osteoclasts and thus promotes catabolic joint remodelling with local bone resorption [11,14]. RANKL belongs to the TNF super family. It is synthesised by mesenchymal cells and T cells [15] and engages its receptor RANK on monocyctic osteoclast precursors. Osteoprotegerin (OPG), a soluble decoy receptor of RANKL that suppresses osteoclast differentiation [16], can modulate the interaction of RANKL with its receptor RANK. RANKL is up-regulated in several experimental models of arthritis as well as in the synovial tissue of patients with RA. Numerous animal studies demonstrated that inhibition of RANK—RANKL interactions by OPG suppresses osteoclastogenesis and reduces bone erosions [17–21].

RANKL is a key player in catabolic joint remodelling as seen in RA. In contrast, RANKL signalling may be suppressed in anabolic remodelling processes including the formation of bony spurs in OA and AS. Indeed, we could demonstrate that inhibition of RANKL by OPG did not halt the development of bony spurs in the rodent models of collagen-induced and adjuvant-induced arthritis [22]. Disturbed RANKL signalling might explain the persistent nature of bony spurs in contrast to transient fracture calluses, which are subject to resorption processes mediated by osteoclasts [23]. Of note, inhibition of RANKL by OPG prevents local as well as systemic bone loss but does not reduce inflammation processes in experimental models of arthritis. In contrast, anti-TNFα or anti–IL-1 treatment, which effectively reduce inflammation, only partially prevent local and systemic bone loss in experimental arthritis [24]. These findings are in line with clinical observations showing good clinical response of patients with AS to TNFα blockers despite progressive worsening by objective parameters.
The promising preclinical results led to the initiation of clinical trials to evaluate inhibition of the RANK-RANKL system in patients with osteoporosis, RA and other conditions associated with loss of bone mass and structure [25]. In the first clinical trials, application of recombinant OPG led to an increase in bone mass and a rapid decline of resorption parameters [26,27]. However, no further clinical studies with OPG were initiated after the first clinical trial with denosumab, a neutralising monoclonal antibody against RANKL. Denosumab showed superior effects compared to OPG and might have a favourable safety profile [28].

Two landmark studies established the anti-resorptive effects of denosumab in post-menopausal osteoporosis [28,29]. In addition to its role in (systemic) osteoporosis, denosumab could be the first anti-bone-resorbing agent to halt focal erosions and osteolysis in inflammatory arthritis [30–32]. A phase II study showed that denosumab significantly inhibited structural damage in RA. Similar to preclinical data on RANKL inhibition, denosumab reduced structural damage of RA without affecting inflammation and clinical disease activity. Thus, anti-RANKL therapy may afford the combination with anti-inflammatory drugs to block inflammatory disease manifestations [33].

**Wnt-Inhibitors dickkopf-1 and sclerostin**

Wnt proteins regulate cell–cell interactions during embryogenesis, cancer and bone homeostasis. Thus far, the canonical, β-catenin-dependent Wnt pathway attracted most attention among several pathways that mediate Wnt signalling. Wnt proteins bind to a receptor complex of lipoprotein receptors (LRP) 4, 5 and 6 and Frizzled proteins. This initiates an intracellular signalling cascade with accumulation and nuclear translocation of β-catenin, which binds co-factors and modulates transcription. Different extracellular proteins can modulate the activity of the Wnt signalling by interacting with LRP4, 5 and 6, Frizzled or Wnt proteins. Among these Wnt-modulating proteins are the Dickkopf (Dkk) proteins and sclerostin [34,35].

![Fig. 1. Key target molecules for cartilage and bone. Cartilage: Fibroblast Growth Factor (FGF)-18 is key growth factor for chondrocytes; Indian Hedgehog controls the formation of hypertrophic chondrocytes, which are less differentiated cells of the chondrocyte lineage; Bone: Receptor Activator of Nuclear factor Kappa B Ligand (RANKL) is essential for differentiation of monocytes into osteoclasts; Cathepsin K (CK) is an osteoclast-specific protease involved in matrix cleavage; Dickkopf (Dkk)-1 is a Wnt blocking factor, which inhibits osteoblast differentiation and bone formation; Sclerostin is a product of the osteocyte and inhibits bone formation.](image)
Wnt signalling as well as its inhibition may have crucial roles in various forms of arthritis. We showed that TNFα induces the expression of the Wnt inhibitor Dkk-1 in both human RA and in murine models of arthritis. In this case, TNFα-mediated Dkk-1 expression promotes bone resorption and blocks bone formation and repair of diseased joints. By contrast, therapeutic scavenging of Dkk-1 by inhibitory antibodies reverses the catabolic pattern of disease into an anabolic one with the formation of new osteophytes. This therapeutic approach demonstrates the possibility to shift the balance from resorption to formation of bone in arthritis, thereby preventing inflammatory bone loss [36].

In animal models, blockade of Dkk-1 activates Wnt signalling and leads to formation of osteophytes, bony spurs and joint fusions, resembling anabolic forms of human arthritis such as AS [36]. Of note, inhibition of Dkk-1 has no inhibitory effect on inflammatory signs of experimental sacroilitis, but significantly reduces bone erosions and osteoclast counts [22]. These findings demonstrate that activation of the canonical Wnt pathway induces new-bone formation and stimulates growth of osteophytes in arthritic joints. Dkk-1 levels in human arthritis confirm these findings: whereas patients with AS display low serum levels of Dkk-1, patients with RA express Dkk-1 levels twice as high as in healthy individuals. As with preclinical models of arthritis, blockade of Dkk-1 might prevent erosions in RA and other forms of human erosive arthritis. In this context, the human anti-Dkk-1-neutralising antibody BHQ880, which can effectively inhibit tumour-induced osteolytic bone disease [37], might be a candidate agent for the treatment of erosive arthritis.

Sclerostin shares many characteristics with the Wnt antagonist DKK-1 and may be another promising target of the Wnt pathway to treat bone and cartilage remodelling. Secreted by osteocytes, sclerostin binds LRP4, 5 and 6 and blocks Wnt-stimulated bone formation [35,38–40]. Consistent with its function as an inhibitor of bone formation, transgenic mice overexpressing human sclerostin display a low-bone-mass phenotype [41,42]. In contrast, sclerostin knockout mice have higher a bone mass with increased bone density and strength [43]. Finally, loss-of-function mutations in the human SOST gene encoding sclerostin leads to increased bone mass in sclerosteosis [44].

In patients with AS and OA, low sclerostin levels may promote anabolic joint remodelling. We showed that the expression of sclerostin is significantly reduced in OA and virtually absent in AS. In contrast, the majority of osteocytes in healthy individuals and patients with RA are positive for sclerostin. Of note, low serum sclerostin levels in patients with AS are associated with the formation of new syndesmophytes. Thus, sclerostin might be a useful biomarker to assess the risk for development of syndesmophytes in patients with AS [45]. Furthermore, replacing sclerostin expression in AS may be another approach to halt anabolic joint remodelling in AS and OA. Conversely, blocking sclerostin might prevent local erosions in RA and systemic bone loss in osteoporosis [43,46].

Wnt signalling is essential for bone homeostasis with anabolic effects upon activation of the canonical Wnt pathway. Its effects in chondrogenic differentiation and cartilage formation, however, are more complex. On the one hand, Wnt can promote chondrogenic differentiation in conjunction with tumour growth factor β [47]. Moreover, conditional chondrocyte-specific deletion of β-catenin leads to a profound degradation of cartilage in mice, suggesting that Wnt activation participates in cartilage homeostasis [48]. On the other hand, Wnt signalling can promote cartilage degradation by activation of catabolic genes in chondrocytes [49]. Furthermore, mice deficient for the Wnt antagonist Frzb-1 display cartilage defects, rendering them susceptible to experimental OA, whereas their ability to form bone is enhanced [50].

These findings indicate that Wnt activation is important for proliferation and differentiation of mesenchymal cells into the chondrogenic lineage, whereas the final differentiation to mature chondrocytes might require the inhibition of Wnt signalling. This could explain the susceptibility of cartilage to degradation in gain-of-function models of Wnt proteins, since full maturation of chondrocytes might be impaired. It could also explain the role of Wnt in numerous processes that require enhanced chondrogenic proliferation as a response to injury, including fracture healing and formation of osteophytes [51–53].

**Cathepsin K**

Bone degradation by osteoclasts comprises two major steps: first, demineralisation of inorganic bone components and, second, removal of organic bone matrix. To demineralise bone, osteoclasts...
secret hydrochloric acid through proton pumps into resorption lacunae. Bisphosphonates, established in the treatment of catabolic bone remodelling in osteoporosis (but not arthritis), interfere with the secretion of hydrochloric acid and prevent bone resorption. In addition to acids, osteoclasts release matrix-degrading enzymes, including lysosomal cathepsin K and other cathepsins [12]. Secreted by osteoclasts, cathepsin K can degrade collagens and other bone-matrix proteins [54]. Consequently, inhibitors of cathepsin K halt catabolic bone remodelling in preclinical models, although they may not completely prevent bone destruction due to the activity of other matrix-degrading enzymes [55].

Beyond inhibition of matrix degradation, cathepsin K inhibition may have anti-inflammatory activity in arthritis. In experimental arthritis, the specific cathepsin K inhibitor NC-2300 showed anti-inflammatory effects, reduced paw swelling and suppressed bone erosions. Further analysis revealed that cathepsin K is involved in innate immune responses via toll-like receptor 9-signalling in dendritic cells. Nevertheless, the exact mode of action and the role of cathepsin K in inflammatory processes of other forms of human arthritis remain unclear [56].

In contrast to bisphosphonates, cathepsin K inhibitors may be effective in the treatment of both systemic osteoporosis and erosive arthritis. Furthermore, cathepsin K inhibitors may have a more favourable side-effect profile than bisphosphonates, since they display less inhibition of osteoclast–osteoblast interactions in bone formation [57]. In this context, one should keep in mind that physiological bone formation requires a tightly controlled balance of both resorption and formation of bone. So far, two cathepsin K inhibitors, balicatib and odanacatib, have entered clinical trials and showed promising results [58–60]. However, the development of balicatib was suspended because of side effects such as skin reactions and elevated parathyroid hormone levels [57].

**Hedgehog signalling**

Intensive studies established the role of hedgehog signalling in skeletal formation during organogenesis [61]. In this context, Indian hedgehog (Ihh), one of the three mammalian homologues of *Drosophila* hedgehog, is essential for chondrocyte and osteoblast differentiation and proliferation. Ihh can either act directly to stimulate osteoblastogenesis or promote osteoclastogenesis via interactions with parathyroid hormone-related peptide (PTHrP) and RANKL [61]. Whereas the balance between bone resorption and bone formation might favour anabolic remodelling during skeletal formation and fracture healing [62], hedgehog signalling might preferentially promote catabolic changes in the adult bone [63] and in OA [64].

Binding of hedgehog proteins (e.g., Ihh) to their receptor Patched (Ptc) initiates the hedgehog pathway. Upon binding of hedgehog proteins, Ptc releases its inhibition on the transmembrane protein smoothened (Smo). This allows Smo to activate the glioma-associated oncogene homologue (Gli) in the cell cytoplasm, which in turn enters the nucleus and modulates transcription processes through interactions with specific DNA-binding elements [65].

Whereas a large body of evidence supports the role of hedgehog signalling in bone formation during organogenesis, little is known about its effects in cartilage and bone remodelling in adults. Recently, Lin and colleagues showed that activation of the hedgehog pathway plays an important role in OA. Samples of human osteoarthritic cartilage displayed activation of hedgehog signalling and mice showed a substantial increase in the expression of the hedgehog target genes in the articular cartilage after surgical induction of OA. Activation of hedgehog signalling in genetically modified mice caused a more severe osteoarthritic phenotype [64]. Since Ihh is a mechanosensitive gene in chondrocytes [66], trauma may up-regulate Ihh owing to abnormal forces on the joint and activate hedgehog signalling in OA.

In patients with OA, articular chondrocytes undergo cellular changes reminiscent of terminal growth-plate chondrocyte differentiation, including hypertrophy and up-regulated expression of COL10A1 [67]. In this context, hedgehog signalling stimulates Runx2 expression, a key factor of chondrocyte differentiation in the terminal growth plate and OA. Of note, Runx2 activates hyperplastic chondrocytes to transdifferentiate into osteoblasts that form new bone. Thus, re-activation of (embryonic) hedgehog signalling pathways may, at least in part, account for cartilage degradation and anabolic bone remodelling in OA. Therefore, hedgehog signalling may be a promising target for prevention and treatment of OA. Indeed, blockade of hedgehog either genetically or pharmacologically
led to a substantial improvement in experimental OA [64]. Of note, clinical trials are evaluating pharmacological blockade of hedgehog signalling by the Smo inhibitor GDC-0449 in patients with cancer. Prior to the initiation of clinical trials in OA, however, further studies should clarify the dual role of hedgehog signalling in cartilage and bone.

**Fibroblast growth factor-18**

Fibroblast growth factors (FGFs) transmit their signals through binding and activation of one of the four FGF-receptor tyrosine kinases [68]. FGF18 activates FGF receptor 2 and 3, known to play major roles in bone and cartilage biology [69–71]. In contrast to its role in the growth plate, where it negatively regulates chondrocyte proliferation and differentiation, FGF18 promotes anabolic processes in articular cartilage of adults [72]. In this context, FGF18 can promote differentiation and growth of articular chondrocytes and stimulate proteoglycan synthesis [72–75]. In a therapeutic approach, FGF18 stimulated chondrogenesis and cartilage repair in a rat model of injury-induced OA. In this model, intra-articular injection of recombinant FGF18 induced a dose-dependent increase in cartilage hypertrophy and overgrowth [76]. Because of the promising results in experimental models of OA, phase II clinical trials will study the efficacy of intra-articular injections of recombinant FGF18 in affected knees of patients with OA.

**Conclusion**

Catabolic and anabolic joint remodelling in arthritis results from an imbalance of degradation and formation of bone and cartilage. In the last few years, central molecular mechanisms have been discovered and potential targets with catabolic (e.g., RANKL and cathepsin K), anabolic (e.g., Wnt proteins, FGF18) and dual effects (e.g., hedgehog) on joint remodelling have been identified. Clinical trials have already evaluated some of these novel targets with promising results. In the future, we will be able to selectively treat the various forms of joint remodelling arthritis.

**References**


1 Ref. 33* Cohen and coworkers path the way for anti-RANKL therapy in RA: This study is the first to demonstrate the efficacy of denosumab in preventing structural damage in RA joints. Ref. 36** The study by Diarra et al. elucidates the central role of DKK-1 and Wnt signalling in different forms of arthritis: Enhanced Wnt signalling may lead to anabolic joint remodelling whereas increased expression of DKK-1 and blockade of Wnt signalling is associated with catabolic joint disease. Ref. 37* In patients with tumor-induced osteolytic disease, Ettenberg and colleagues demonstrate for the first time that blockaded of DKK-1 by the neutralizing antibody BHQ880 effectively inhibits bone degradation in humans. Ref. 45* This interesting study points out the potential role of sclerostin as a novel biomarker in AS. Sclerostin may be helpful to estimate the risk for development of syndesmophytes. Ref. 56** As shown by this outstanding study, cathepsin K is not only involved in matrix degradation but also promotes inflammation via TLR-9 in experimental arthritis. Thus, cathepsin K inhibitors might block matrix degradation as well as decrease inflammation in human arthritis. Ref. 64** This landmark study establishes the role of hedgehog signalling in OA. Lin and coworkers demonstrate that altered hedgehog signalling can increase the susceptibility for the development of OA and might contribute to anabolic remodelling of OA joints.


