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Targeting subchondral bone for treating osteoarthritis: what is the evidence?

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Over the past few decades, significant progress has been made with respect to new concepts about the pathogenesis of osteoarthritis (OA). This article summarises some of the knowledge we have today on the involvement of the subchondral bone in OA. It provides substantial evidence that changes in the metabolism of the subchondral bone are an integral part of the OA disease process and that these alterations are not merely secondary manifestations, but are part of a more active component of the disease. Thus, a strong rationale exists for therapeutic approaches that target subchondral bone resorption and/or formation, and data evaluating the drugs targeting bone remodelling raise the hope that new treatment options for OA may become available.

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Introduction

Bone remodelling is continuously maintained through a tight equilibrium between osteoblast activity responsible for the bone formation through the synthesis of bone matrix and an

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osteoclast activity accountable for degrading the bone microenvironment. The equilibrium between the activities of these two cells preserves the mineral component of calcium and phosphorous molecules. Bone remodelling includes several distinct phases, namely the activation, resorption, reversal and formation processes [1–3]. In this cycle, several components, including the specific cells, the osteoblasts, osteocytes and osteoclasts; inorganic non-collagenic substances, such as proteoglycans; and a collagenic component of which collagen type I is the major constituent, are all closely modulated. Mechanical force and stress induce bone alterations, in which osteoclast precursors and mature osteoclasts are recruited from the circulation to the bone-remodelling unit, thereby initiating the mineralised matrix resorption. Following this process, osteoclasts undergo apoptosis, leaving an opening for osteoblasts to invade and build up a new extracellular matrix.

Osteoclasts are derived from the haematopoietic progenitors of the monocyte–macrophage lineage and can be distinguished by their multinucleated giant cell appearance together with specific features such as polarised morphology and an apical differentiation adjoining the calcified matrix in resorption (ruffled border) [1–3]. They are responsible for the acidification of the extracellular matrix followed by the release of proteolytic enzymes, which leads to the breakdown of the bone matrix. The osteoblasts are derived from bone marrow cells and are responsible for the production of new matrix [1–3].

Osteoarthritis

Several tissues of the joint, including the cartilage, the synovial membrane and the subchondral bone, play significant roles in the development/progression of osteoarthritis (OA) pathology [4]. Although articular cartilage breakdown is a major characteristic of OA and synovial membrane inflammation also actively participates in the progression of the joint tissue lesions, the precise mechanism initiating its degradation is still unknown. During the initiation/progression of OA, subchondral bone is the site of numerous dynamic morphological transformations due to an altered osteoblast metabolism, which are part of the pathological process. Most importantly, subchondral bone remodelling in OA progresses from increased bone resorption early on to bone accretion, resulting in sclerosis of this tissue. In a primate model of spontaneous OA, increased osteoid volume is often more severe than cartilage changes [5]. Moreover, in this animal model, the severity of cartilage fibrillation and loss generally exceeds bone changes only in advanced OA. Although it has been shown in some induced OA animal models, which allow chronological analysis of the disease progression, that the subchondral bone changes precede cartilage changes [6–9], this has yet to be clarified in humans.

However, *in situ* structural changes in subchondral bone during the course of OA can now be readily observed using imaging techniques. Indeed, magnetic resonance imaging (MRI) revealed the presence of bone marrow lesions (hypersignal), which increased in size gradually over time [10–12]. Using this technology, the presence of oedema-like lesions in subchondral bone marrow and bone attrition were found to be strong indicators of bone turnover indices as well as structural deterioration in knee OA.

Moreover, a study performed using the anterior cruciate ligament (ACL) transection OA dog model showed that, in general, bone marrow hypersignal as assessed by MRI is topographically associated with cartilage lesions as seen macroscopically [13]. Data also revealed that the loss of cartilage volume/thickness and the deterioration of the subchondral bone structure were interdependent in longitudinal studies in knee OA patients [12,14]. The loss of cartilage, subchondral bone alterations and osteopenia of the underlying trabecular bone were all correlated in some of these patients. Using radiographs in a cross-sectional study, Beuf et al. [15] also demonstrated that the loss of trabecular bone in the femurs of OA patients correlated with the severity of the disease as assessed by the radiography-based Kellgren–Lawrence scale. Interestingly, such hypersignal bone lesions were recently evaluated histologically and appear to correspond mainly to fibrosis, myxoedematous degeneration and/or cellular infiltrate of the bone marrow [16]. Altogether, these findings strengthen the hypothesis that the subchondral bone changes may play a role in the genesis of cartilage lesions.

Subchondral bone remodelling

Early phase of OA: subchondral bone resorption

It is believed that alterations in subchondral bone activity occur quite early in the OA process. Although bone sclerosis is considered a hallmark of OA, subchondral bone indices of resorption were found in patients with progressive knee OA. This suggests that such alterations in the OA subchondral bone have an impact on both the quality and quantity of this tissue. The early bone resorption features observed in OA patients were carefully assessed in a subset of knee OA patients from the Chingford study [17] by measuring markers of bone resorption, such as urinary N-terminal type I collagen telopeptides (NTX) and C-terminal type I collagen telopeptides (CTX), at three different time points. Patients with progressive worsening of the knee showed bone resorption but those with non-progressive OA did not.

Correlation between in vivo findings in animals and human OA patients

Animal models of OA show that the indices of bone resorption are increased early in the disease process and that bone formation is a relatively late phenomenon. In a guinea pig model, bone densitometry evaluation following meniscectomy revealed typical variations in bone metabolism with early resorption of subchondral bone followed by increased bone density [18]. This concurs with findings from other animal models, such as the ACL dog and rat OA models, in which were observed, at an early stage of the disease process, increased subchondral bone resorption with trabecular thickness reduction and an increased number of osteoclasts, as well as increased production of catabolic factors including cathepsin K and matrix metalloproteinase (MMP)-13 [6–9].

Later phase of OA: subchondral bone sclerosis

As mentioned above, studies have also demonstrated that as the disease progresses, or at a later stage of OA, subchondral bone becomes sclerotic. Using quantitative microfocal radiography, Buckland-Wright et al. [19,20] showed that the changes in OA joints also involve thickening of the subchondral cortical plate. In the ACL dog model of OA, Brandt et al. [6] reported that, at a later stage of the disease, 54 months after OA induction, there was a marked increase in the volume of subchondral bone with active bone formation. Studies in OA of the knee and the hand reported that the scintigraphic abnormalities correlated with the osteocalcin concentration in the synovial fluid, which is a marker of bone formation, and serum osteopontin, a bone matrix protein, were also elevated [21–23]. Gevers and Dequeker also showed elevated serum osteocalcin levels in women with hand OA, and elevated osteocalcin in cortical bone explants [24]. Moreover, levels of growth factors such as insulin-like growth factor (IGF)-1 and -2 and transforming growth factor (TGF)- β 1 are also higher in samples of iliac crest bone of patients with OA [25]. Considering that this bone is a non-weight-bearing site and at some distance from joints, this would suggest a generalised dysfunction in bone metabolism.

Subchondral bone sclerosis in OA appears to be due to an increase in material density, and not mineral density

Stiffness and bone mineral density (BMD) are not uniform in OA bone [26]. The bone closest to the articular cartilage has the greatest effect on cartilage integrity, with variations in stiffness and BMD probably causing more damage to cartilage than any other parameters under normal conditions [27,28]. Although OA at a later stage demonstrates a thickening of subchondral bone, explants of the femoral heads of OA patients at autopsy revealed a pattern of low mineralisation compared with that of normal [29,30]. Hence, the apparent increase in bone density in OA may be because of an increase in material density, and not mineral density [31–33]. Indications of altered mineralisation and abnormal metabolic subchondral bone can be inferred from the increased osteoid matrix in this OA bone tissue [24,32,34,35]. As the osteoid matrix is primarily composed of an abundant collagen type I matrix, it was therefore of no surprise that this collagen type in the trabecular bone of the femoral heads of OA patients was found to be increased [31,32], along with abnormal collagen type I fibres, as evidenced by an increased ratio of α 1 to α 2 chains in OA compared to normal trabecular subchondral bone [33]. Data

also reported a two- to threefold increase in the expression of *COL1A1* chains of type I collagen with no variations in *COL1A2* expression in OA subchondral bone osteoblasts, leading to an increase in the production of type I collagen $\alpha 1$ chains [36]. Together with the reduced number of cross-links in OA bone tissue [32], this could explain the reduction in bone mineralisation. Moreover, data also showed an increase in osteocalcin and alkaline phosphatase in human OA subchondral bone both *in vivo* [24] and *in vitro* [37,38]. Hence, the early marker (alkaline phosphatase), terminal differentiation marker (osteocalcin) and the mineralisation of OA subchondral bone osteoblasts are altered.

Role of cells/factors in abnormal subchondral bone metabolism in osteoarthritis

Mesenchymal cells

Aspden et al. [39] proposed that the formation and activity of mesenchymal stem cell (MSC) precursor cells in OA skeletal tissues could be modified by systemic and/or local factors. One possibility was that one or more intrinsic cell functions are altered or that abnormal lipid metabolism leads to OA. In agreement with this concept, the same group showed abnormal lipid composition of trabecular bone from OA patients [40]. Moreover, they postulate that, if this is the case, the fate of MSCs in OA should be altered. Further data indeed revealed that the osteogenic potential of bone marrow cells from OA patients was increased compared with that from normal, whereas both the chondrogenic and adipogenic potential of these cells were blunted [41].

In vivo, the altered bone collagen matrix in OA bone tissue may contribute to induce the MSCs to differentiate into osteoblasts. The altered fate of MSCs may also result from an exposition to leptin, because it promotes the differentiation of these cells into osteoblasts while it inhibits adipocyte maturation [42]. This latter effect could also be linked with the collagenous extracellular matrix because leptin also directly stimulates the production of osteoblast markers and collagen [43]. Recent studies have also focussed attention on the potential direct contribution of mature osteoblasts/osteocytes to the recruitment and fate of MSCs via the Wnt signalling pathway. Indeed, the control of adipogenesis, osteogenesis and chondrogenesis in bone marrow may be regulated locally by Wnt agonists and antagonists produced by the mature osteoblast/osteocytes [44,45]. Osteocytes also contribute to local control of bone resorption through the production of the Wnt antagonist, sclerostin (SOST) [46,47]. It is, therefore, also of note that OA osteoblasts produce abnormal levels of Wnt antagonists Dickkopf (DKK)-2 and SOST, which are involved in terminal osteoblast differentiation and bone resorption, respectively [48,49]. Hence, *in vivo* alterations are likely due to changes in abnormal cellular metabolism and not to changes in systemic regulation.

Moreover, the Wnt/ β -catenin signalling pathway is a normal physiological response to mechanical loading in bone [50], and hydrostatic pressure promotes Wnt10b and Wnt4 expression in early-osteinduced MSCs [51]. Therefore, biomechanical forces in OA may have an impact on the biological response of MSCs and osteoblasts, and in the presence of an abnormal extracellular matrix, these forces would be improperly transmitted within the bone microenvironment.

Wnt

The key role of Wnt in the regulation of bone mass was discovered recently by identifying both activating and loss-of-function mutations in LRP5. Indeed, a single modification of an amino acid in the extracellular domain of LRP5 was linked to high bone mass with resulting weak activation of the β -catenin pathway [52–55]. Conversely, a loss-of-function mutation identified in both humans and mice results in low bone mass [56,57]. A weak association between haplotypes of the LRP5 gene indicates a risk factor for OA [58], and a single nucleotide polymorphism in secreted frizzled-related protein 3 (sFRP3) was identified in a group of females with hip OA [59], but not in knee OA. sFRP3 is an antagonist of the Wnt pathway and the polymorphism reduces the ability of sFRP3 to antagonise Wnt signalling, thereby suggesting that Wnt signalling might be elevated in OA. However, sFRP3 can decrease the proliferation and increase the differentiation of mature osteoblasts via a β -catenin-independent pathway [60].

Among the antagonists of Wnt signalling, DKK1 and DKK2 play key roles in osteoblast proliferation, differentiation and mineralisation. DKK1 is a master regulator of osteogenesis [61–63] whereas DKK2 acts as a fine-tuning regulator of osteoblast proliferation, terminal differentiation and mineralisation [49]. Lastly, SOST is an osteocyte-derived negative regulator of bone formation. A loss-of-function mutation of SOST was identified in sclerosteosis patients [64] who show progressive bone thickening and generalised osteosclerosis. SOST was believed to be a bone morphogenic protein (BMP) antagonist because it inhibits BMP-stimulated bone formation, yet it cannot antagonise all BMP responses [47,65]. Moreover, Wnt proteins induce BMPs, a mechanism inhibited by SOST, indicating that SOST may be a canonical Wnt signalling inhibitor, and that BMP activity is required downstream of a Wnt stimulus [65–68]. Of note, our group recently reported [48] that a member of the DKK family, DKK2, a Wnt antagonist, was responsible for the altered phenotype of human primary OA osteoblasts.

IGF and TGF- β 1

IGFs are important growth factors that regulate bone formation. OA subchondral bone osteoblasts produce variable total IGF-1 levels and less IGF-binding proteins compared with normal [69]. This results in higher levels of free IGF-1 that could promote bone remodelling and increase bone stiffness, a situation that exacerbates cartilage matrix degradation. TGF- β 1 and IGF-1 are also involved in matrix deposition and turnover, with TGF- β 1 stimulating matrix synthesis, but, as mentioned above, leading to an abnormal type I collagen α chain ratio and collagenase activity, whereas IGF-1 inhibits matrix degradation in bone cells [70]. It is worth mentioning that a study in mice showed that intra-articular injections of TGF- β into the knee joint induced OA-like changes [71] and that blocking endogenous TGF- β production during experimental OA prevents osteophyte formation [72]. The localised effect of TGF- β and IGF-1 may be linked to an abnormal response to leptin by OA subchondral bone osteoblasts. This may occur as the expression of leptin stimulates TGF- β and IGF-1 in joint tissues [73].

Recent evidence further indicates that abnormal TGF- β 1 levels can directly affect subchondral bone tissue quality in OA by altering mineralisation. Indeed, Couchourel et al. [36] recently demonstrated that OA subchondral bone osteoblasts fail to mineralise normally, whereas correcting their endogenous TGF- β 1 levels by sustained inhibition enhanced their mineralisation. Moreover, these authors [36] also showed that inhibiting TGF- β 1 expression induced a correction of the abnormal ratio of type I collagen α 1 to α 2 chains observed in OA subchondral bone osteoblasts.

OPG/RANK/RANKL

In the context of bone resorption, a molecular triad composed of osteoprotegerin (OPG), receptor activator of nuclear factor- κ B (RANK) and RANK ligand (RANKL), members of the tumour necrosis factor superfamily, has been described as a key cytokine system involved in the differentiation and function of osteoclast cells [74–78].

In the bone remodelling process, the factors of this triad are closely linked to each other. RANKL is expressed in either membranous or soluble form, primarily by the osteoblastic lineage cells, and is essential for mediating bone resorption through osteoclastogenesis and the activation of mature osteoclasts. RANKL stimulates osteoclastogenesis and osteoclast activity by binding to the cell surface receptor RANK, located on precursor and mature osteoclasts. The binding of RANKL to the extracellular RANK domain leads to the activation of specific signalling pathways involved in the formation and survival of osteoclasts, hence bone resorption. The third protagonist, OPG, is secreted by the stromal cells and other cell types, including osteoblasts, and acts as a soluble decoy receptor for RANKL. OPG, by interacting with RANKL, inhibits the binding of RANKL to RANK, thereby blocking RANK activation and subsequent osteoclastogenesis.

Investigation of this molecular triad in human OA subchondral bone osteoblasts revealed that these cells could be discriminated into two subgroups based on their having either a low or a high OPG/RANKL ratio [79]. Moreover, investigation of the ability of each subgroup to induce osteoclast differentiation demonstrated that the OA subchondral bone osteoblasts with a low OPG/RANKL ratio induced a greater number of mature osteoclasts compared with the high OPG/RANKL ratio OA osteoblast subgroup [79]. Hence, these OA subpopulations could favour bone resorption or bone formation,

respectively. Interestingly, *in situ* histological evaluation of the subchondral bone in each subgroup of OA patients compared with normal individuals suggested that the OA subgroup with a low OPG/RANKL ratio showed reduced subchondral bone thickness, and the OA subgroup with high OPG/RANKL ratio showed increased thickness [79].

Ephrin system

Recently, a system composed of the ephrin ligands and their specific receptors Eph has been described for its ability to control bone remodelling [80]. Two subclasses of Eph receptors have been defined according to their preferred ligand specificity, namely type A receptors (EphA) which bind to ephrins A, and type B receptors (EphB) which bind to ephrins B. However, there are some exceptions in which, for example, an ephrin B binds to an EphA receptor. The two populations of ephrins differ in their anchorage: ephrins A are composed of a GDI anchor, while ephrins B possess a single trans-membrane domain.

Both ephrins and Eph receptors are cell-membrane-bound proteins and their interaction leads to a bidirectional (osteoblast/osteoclast) Eph/ephrin signalling (Fig. 1). In this particular situation, signalling through EphB receptors is considered forward and, through ephrin B ligands, reverse signalling [80]. Interestingly, it was recently demonstrated that ephrin B2, mainly expressed by osteoclasts, and its specific receptor EphB4, expressed by osteoblasts, are involved in the control of bone homeostasis in which the EphB4 forward signalling favours an osteoblast differentiation process, whereas the reverse

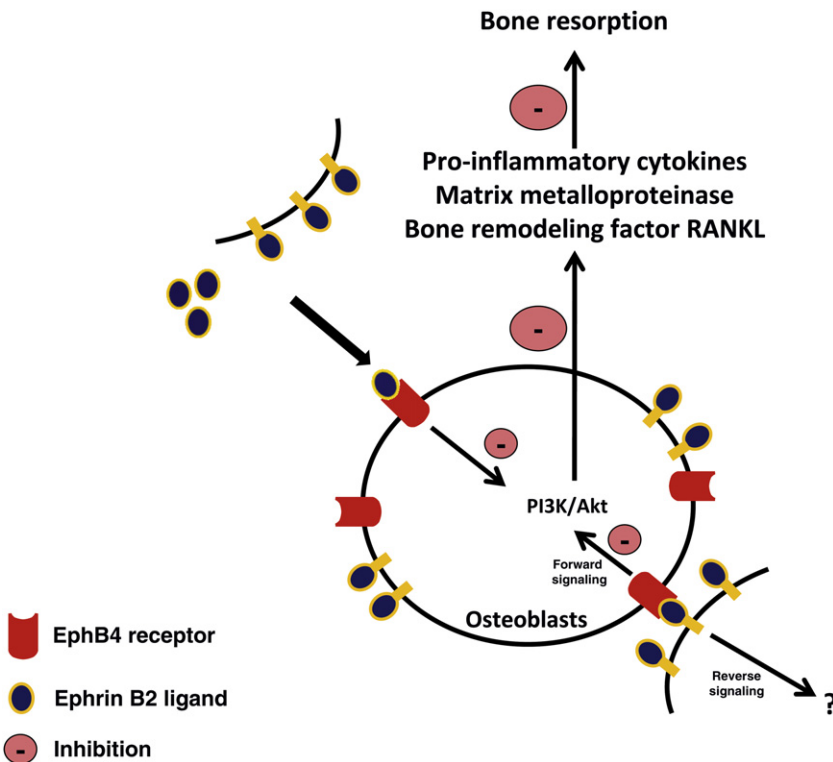


Fig. 1. Interaction between the EphB4 receptor and ephrin B2 ligand leads to both a forward and a reverse signaling. The signal through the EphB4 receptor is considered a forward signal whereas the reverse signaling is through the ephrin B2 ligand. In OA subchondral bone osteoblasts, the activation of EphB4 receptor by ephrin B2 results in a decreased activation of PI3K/Akt which, in turn, inhibits some pro-inflammatory cytokines and matrix metalloproteinases as well as RANKL, all of which are involved in the remodeling process of the OA subchondral bone [81].

signalling through ephrin B2 ligands leads to an inhibitory effect on the osteoclast function. The overall outcome of such an interaction favours bone formation [80].

As mentioned above, OA subchondral bone osteoblasts can be discriminated as pro-resorptive or pro-formative. Interestingly, these two subgroups were also found to differentially express EphB4 receptors and ephrin B2. Those showing pro-resorptive properties showed a higher level of EphB4 receptors and ephrin B2, while those in a pro-formation phase had EphB4 receptor and ephrin B2 ligand expression levels similar to the normal subchondral bone osteoblasts [81]. Although not yet tested, the increased levels of these factors in the pro-resorptive osteoblasts would suggest an attempt by these cells to regulate their resorption activity. In the other OA osteoblast subpopulation, an increase in these factors would not be required, as these cells are in a pro-formation phase. This concurs with further data showing that in pro-resorptive osteoblasts, treatment with ephrin B2 ligands induced a reduction in the bone resorption process as well as in various catabolic mediators, including the inflammatory factors interleukin (IL)-1 β and -6; the MMPs -1, -13 and -9; and RANKL [81]. This process appears to occur through an inhibition of the PI3K/Akt signalling pathway.

Other factors

OA subchondral bone osteoblasts were shown to produce less cAMP in response to parathyroid hormone (PTH) stimulation [37], and they have fewer PTH receptors [82]. Since PTH is a negative regulator of collagen synthesis, this could then lead to altered type I collagen production in OA bone tissue [83,84].

An abnormal degradation of the extracellular matrix could also potentially lead to an alteration in OA subchondral bone tissue, as it orchestrates interactions of osteoblasts with neighbouring cells, and changes in matrix composition may alter cell function and communication. In this context, two MMPs, -2 and -9, were found to be elevated in proximal cancellous bone tissue isolated from the femoral heads of OA patients [32], a situation that may be linked to abnormal collagen matrix deposition.

Interaction between subchondral bone and cartilage

It is currently suggested that factors produced locally in subchondral bone can act on chondrocytes in the articular cartilage. However, the question arose as to how these factors could reach the overlying cartilage. The possibility of diffusion of molecules/factors from the subchondral bone to the articular cartilage was evaluated when it was observed that the elastic modulus (stiffness) of the subchondral bone was reduced locally due to an increase in vascularisation and in the remodelling rate [85]. These changes would then lead to reactivation of the secondary ossification centre and a decrease in cartilage thickness [86], suggesting that the altered subchondral bone remodelling or turnover would initiate cartilage degradation. This is possible as clefts and channels in the tidemark were seen early on in OA as well as fatigue microcracks in articular cartilage [87–91].

Nutrients must enter articular cartilage either from the surface, through the synovial fluid, or from the underlying subchondral bone, because healthy cartilage is considered avascular. Although it was initially believed that synovial fluid was the only nutrient route due to the absence of an anatomical barrier and because the cartilage cannot survive when not nourished by the synovial fluid [92], this concept was recently challenged. Indeed, cartilage can degenerate even when in contact with normal synovial fluid; hence, nutrient supply through the subchondral bone may seem obvious. Conversely, it can be argued that the dense calcification in the basal zone of normal articular cartilage constitutes an insurmountable barrier to solute and fluid diffusion. Interestingly, when cartilage was deprived of contact with subchondral bone for a long period in the baboon, the cartilage degenerated as in OA [93]. Therefore, both routes of nutrient entry may co-exist.

In the spontaneous OA guinea pig model, the severity of articular lesions and progression of the disease were also increased in animals with the highest bone turnover rate [94]. Hence, the state of OA subchondral bone at any given time point would be less important than tissue homeostasis and turnover to the appearance and progression of OA, which indicates the dynamic nature of the disease. The response to the biomechanical alterations of the subchondral bone tissue at any time point would

then induce local responses that contribute to thinning of the articular cartilage. This would then further contribute to shear stress and lead to complete cartilage loss.

Nonetheless, what triggers the initial cartilage damage is still speculative. As mentioned above, some studies have indicated that subchondral bone changes precede and may be responsible for the evolution of cartilage lesions [29,95,96]. Others have indicated that subchondral bone changes would only be secondary to cartilage degradation [97,98]. Although the pathways involved in cross-talk between subchondral bone and cartilage remain largely unknown, some factors synthesised by subchondral bone cells are capable of inducing metabolic changes in the cartilage.

Potential factors in subchondral bone/cartilage cross-talk

Bone produces a number of factors that are involved in both tissue remodelling and the modulation of cartilage catabolism. Hence, subchondral bone tissue may, through the production of cytokines, growth factors and eicosanoids, induce OA cartilage degradation [98–100]. Interestingly, in addition to the subchondral bone cells, the OPG/RANK/RANKL molecular triad has also been observed to be expressed and produced by another articular cell, the chondrocyte. Recent findings showed that human chondrocytes express and produce these factors and that treatment of these cells with OPG results in increased levels of two catabolic factors involved in cartilage pathophysiology, MMP-13 and proteinase-activated receptor (PAR)-2 [101]. However, treatment with RANKL did not show any effect. This could reflect the fact that RANK was shown to be produced by only 29% of OA chondrocytes localised throughout human cartilage [101].

Ephrin B2 and its specific receptor EphB4 were also found to be expressed by human chondrocytes and treatment with ephrin B2 also positively impacts on the abnormal metabolism of OA cartilage by inhibiting IL-1 β , -6, MMP-1, -9, -13 and PAR-2 levels and increasing collagen type II gene expression [102].

The hepatocyte growth factor (HGF) may also be involved in the cross-talk between the two tissues. Indeed, HGF is expressed and produced in human subchondral bone osteoblasts and at a higher level in OA, whereas only the protein, not the gene, is detected in articular cartilage in OA patients, and only in the lower intermediate and deep layers [103–105], thus suggesting that subchondral bone osteoblasts may be responsible for the HGF found in OA cartilage. Moreover, HGF induces MMP-13, an enzyme involved in OA cartilage degradation [106] and mostly present in the intermediate and deep layers of articular cartilage [107], the same site where HGF is detected.

Bone markers in OA

One of the fundamental problems with OA was the absence of validated markers that could predict the disease or permit follow-up of affected individuals. Indeed, although OA is a prevalent chronic health condition, its diagnosis is based on the clinical assessment and/or conventional radiography that is considered the gold standard, with radiographic changes such as joint-space narrowing and osteophyte formation that appear rather late during the disease process. This radiography approach has inherent limitations, one of which is that it can only detect an advanced stage of the disease in patients and cannot be used to determine early or subtle changes over time. However, imaging methods other than radiography were recently used to detect markers or risk factors for this disease. The literature reveals that MRI is very useful for the assessment of disease activity and the determination not only of disease progression but also of early events [10,11]. In this context, meniscal lesions and bone marrow oedema were demonstrated to be important risk factors for OA progression [12,108].

Individual markers were measured in a number of clinical studies and some indicated correlation with disease activity or progression, but not initiation of OA. These included studies measuring degradation and/or turnover products from cartilage, bone or the synovial membrane [109–114]. Indeed, the focus has been drawn away from the initial determination of individual markers such as hyaluronic acid (HA), C-telopeptide of type II collagen (CTX-II), cartilage oligomeric matrix protein (COMP), sulphate (KS)-5D4, urinary type II collagen-related epitopes, TGF- β 1 and type II procollagen carboxy-propeptide (CPII), which are all excellent to determine patients at greatest risk of progression or to discriminate patients with more severe OA from those with less severe OA [109,110,115]. However,

these markers could not discriminate either initiation or activity of the disease *per se*. More sophisticated determination of chondroitin sulphate (CS) epitope WF6 was also done in both OA and rheumatoid arthritis patients. However, as the levels of both HA and WF6 were elevated in patients with these two diseases, a strict correlation with disease activity was not fully validated [116]. As the involvement of bone tissue was also becoming recognised in the initiation and/or progression of OA, biochemical markers of bone turnover were also determined in some clinical studies. More specifically, serum C-terminal cross-linked telopeptide of type I collagen (CTX-I), serum osteocalcin, glucosyl-galactosyl-pyridinoline (Glc-Gal-PYD) and the cartilage marker CTX-II as determined in the MRC Hertfordshire Cohort [117] indicated a strong association with knee OA as assessed by the Kellgren–Lawrence scale. By contrast, no individual association could be found for serum osteocalcin or serum CTX-I.

Whereas these studies were very useful in identifying targets to monitor patient outcomes in clinical studies and progression of the disease in response or not to specific treatments, individual markers, such as those reflecting cartilage degradation, synovial inflammation or bone turnover, were not very informative on their own, and they failed to determine the disease initiation. In recent years, however, the trend has shifted from assessments of individual markers to uncovering clusters of markers that may correlate with disease progression and activity. Indeed, it was recently demonstrated, using an approach based on the association of selected biochemical markers combined into a single factor, that a combined panel of biochemical markers showed a stronger association with OA than any individual markers [118]. Moreover, their analysis also suggested that markers identifying patients with osteophytes overlapped those with a high Kellgren–Lawrence score while markers for BMD of the subchondral bone were uncovering a totally different group, hence raising the possibility that although osteophytes and subchondral BMD are associated with OA, they may have underlying biological differences. Likewise, a study by Meulenbelt et al. [119] also demonstrated that combining biochemical markers was more sensitive at detecting subtle changes in different OA patients, that cartilage and bone turnover markers were more sensitive at detecting OA, particularly hip OA, whereas markers of inflammation correlated with knee OA, and that the changes in cartilage turnover markers correlated with hand and spine OA. Other studies also suggested an imbalance between bone formation and bone resorption indices in OA patients and, more specifically, identified increases in OPG and soluble RANKL (sRANKL). These increases actually led to an increased sRANKL/OPG ratio, which should favour bone resorption in OA patients, and correlated with disease severity [120].

Table 1
Individual markers of OA progression and initiation.

Markers of OA progression [109–113,115–117]
Hyaluronic acid
C-telopeptide of type II collagen (uCTX-II)
Cartilage oligomeric matrix protein
Keratan sulfate KS-5D4
Urinary type II collagen-related epitopes (uC2C)
Transforming growth factor β -1
Type II procollagen carboxy-propeptide
Chondroitin sulfate epitopes WF6
Serum C-propeptide of type II procollagen (sCPII)
Markers of pre-ROA* [122]
uCTX-II:sCPII
uC2C:sCPII
Miscellaneous markers [109,117,120]
Serum C-terminal crosslinked
Telopeptide of type I collagen (CTX-I)
Serum osteocalcin
Glucosyl-galactosyl-pyridinoline
OPG;OPG/RANKL ratio

*ROA: radiographic OA.

However, these studies still failed to uncover any specific markers that could relate to early events and therefore be predictive of OA initiation. This was recently challenged by a new study that identified biochemical markers that may actually accompany early OA and precede radiographic changes by almost 10 years. Indeed, Ling et al. [121] used a microarray platform that simultaneously tested 169 proteins relevant to inflammation, cell growth, activation and metabolism in a case-control study. They identified 16 proteins that were different between OA patients and control subjects who had not developed OA. Four of these markers associated with cell activation, inflammation and bone collagen degradation were already different 10 years prior to radiographic classification of the individuals as having OA and were still different at the time of diagnosis. Six more proteins were clearly associated with subsequent development of OA. A recent study by Cibere et al. [122] also reported that individual markers could be useful nevertheless, that the usefulness of these markers depended on the stage of OA and that ratios of a combination of markers were more informative.

Therefore, although studies are indicating that individual markers may be suitable for following patients or determining the outcome of specific intervention (Table 1), clusters of biochemical markers appear to be a much more promising avenue for identifying patients at the initiation of the disease (Table 2). These markers alone or in combination with MRI data would then provide a means to identify those patients at risk much earlier, with the possibility of intervention. The prospect of detecting those patients at risk of developing OA may then be useful for testing disease-modifying OA drugs as they become available.

Therapeutics targeting osteoarthritic subchondral bone

The findings demonstrating a remodelling process in the subchondral bone during the OA pathophysiology has raised the question as to whether factors capable of influencing bone metabolism could be used as therapeutic strategies targeting this tissue in OA patients. In this context, a number of

Table 2

Clusters of markers used together for the detection of OA.

Cluster of markers of pre-ROA* [121]

Matrix metalloproteinase (MMP)-7
Soluble vascular adhesion protein (sVAP)-1
Interleukin (IL)-15
Plasminogen activator inhibitor (PAI)-1
MMP-2
D-dimers (DD)5 and DD6
Eotaxin-2 (Eot-2)
Intracellular adhesion molecule (ICAM-1)
P-selectin

Cluster of markers of early ROA [121]

B-lymphocyte chemokine (BLC)
6-chemokine (6Ckine)
Macrophage inhibitory protein (MIP)1 α
IL-1 α
IL-2
Fibroblast growth factor (FGF)-7
Insulin-like growth factor (IGF) binding protein-2
Granulocyte macrophage colony stimulating factor (GM-CSF)
Neurotrophin-4 (NT-4)
ICAM-3
Vascular endothelial (VE)-cadherin

Cluster of markers of ROA and other indications [119]

uCTX-1; u-CTX-II; OC; Glc-Gal-PYD Bone turnover
HsCRP; high BMI Inflammation

*ROA: radiographic OA.

studies, most using OA animal models, have explored the effects of drugs/agents that can modulate bone metabolism, but to date only a few have undergone human clinical trials.

Future prospects

OPG and anti-RANKL

As mentioned above, the factors OPG and RANKL are highly involved in the control of bone biology. The pro-resorptive effect of RANKL on the osteoclastogenesis process could therefore be targeted by the use of either OPG or an anti-RANKL antibody. A recent study carried out in an experimental mouse model of OA revealed, upon OPG administration, reduced cartilage degradation through an effect on trabecular bone [123]. In the same line of thought, data from an OPG transgenic mouse model of OA suggested that the *in vivo* beneficial effect of the administration of OPG could be due to its capacity to bind tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), an inducer of chondrocyte apoptosis [124]. However, data generated from an *in vitro* study in which treatment of human OA chondrocytes with OPG enhanced two catabolic factors involved in the pathophysiology of the disease [101] suggest that targeting the factor that mediates the effect of OPG, for example, by inhibiting RANKL, could lead to a new therapeutic approach against OA. Such inhibition of RANKL has been proposed as a therapeutic approach in some osteolytic diseases [125,126]. It is thus plausible that therapies interfering with both cartilage and subchondral bone simultaneously could block, or at least attenuate, the progression of OA. The potential of RANKL inhibition as a disease-modifying drug is thus very appealing. However, only appropriate clinical trials will be able to provide the information needed to address this important question.

Ephrin B2/EphB4 receptors

In vitro findings showing that ephrin B2 treatment in human OA subchondral bone and chondrocytes could positively impact on the abnormal metabolism of these diseased cells [81,102] suggest that enhancing this system could lead to a protective effect on the structural changes in these OA tissues.

Drugs/agents that target bone remodelling

Bisphosphonates

Following the line of thought that an active remodelling process occurs in OA subchondral bone and that such phenomenon could contribute to the cartilage degeneration and disease progression, anti-resorptive substances such as bisphosphonates would seem well suited against the progression of OA. Bisphosphonates are among the most commonly used drugs for the treatment of diseases involving excessive resorption of bone, such as osteoporosis.

Studies using different animal models were first conducted with alendronate. In the rat ACL transection model, alendronate demonstrated chondroprotective effects with osteophyte formation inhibition and reduction in cartilage degradation biomarkers [7]. However, different results were obtained with this drug in the spontaneous guinea pig model, in which alendronate increased the bone mineral content and density, which was associated with the acceleration of cartilage degradation [127]. Interestingly, another bisphosphonate, risedronate, when tested in the same model, showed a positive effect on the progression of OA, reducing the size and severity of cartilage lesions and limiting osteophyte formation [128]. In this latter study, a comparison between risedronate and alendronate revealed the former to be more effective. Furthermore, a comparative study among different bisphosphonates revealed that only the compounds containing nitrogen and pyridinyl side chains positively affect cartilage structure [129]. In the ACL dog model of OA, the morphological changes that take place at the subchondral bone level, particularly in the early phase of the disease, are predominantly resorptive in nature. A study in this model using NE-10035, a bisphosphonate administered through subcutaneous injection, showed that over 3 months following surgery, the prophylactic treatment effectively reduced the turnover and resorption of subchondral bone in the OA joint [130]. However, the treatment had no effect on osteophyte formation or the severity of cartilage changes.

In a phase II clinical trial in knee OA patients, oral treatment with risedronate was shown to decrease CTX-II production [131]. However, in a phase III trial, risedronate failed to show any disease-modifying effect in such patients [132]. The reasons for the failure of such trials could be multiple. One reason may be that for treatment to be effective the drug should be administered during the early stages of OA. Moreover, the imaging technology (X-ray) used in these investigations may not have been optimal for assessing a disease-modifying effect. Indeed, data have shown that a few thousand OA patients would be required to demonstrate a statistically significant effect using X-ray technology [132].

Strontium ranelate

Strontium ranelate is a newly developed drug that induces an increase in bone mass and an amelioration of bone architecture. Strontium ranelate is an approved treatment for osteoporosis, reducing the risk of both vertebral and hip fractures. Strontium ranelate acts on both bone formation indices and bone resorption indicators. Indeed, strontium ranelate prevents trabecular bone loss, decreases bone resorption and increases or maintains bone formation in the ovariectomised rat model or in rats subjected to hindlimb immobilisation [133,134]. Strontium ranelate also reduces bone resorption and enhances bone mass and strength in mice, rats and monkeys [135–137]. In *in vitro* studies, strontium ranelate also directly reduced osteoclast differentiation and resorption, whereas it increased pre-osteoblast replication and differentiation [138–142]. Preclinical studies in normal and osteopenic animals provided evidence that the dissociation of bone formation and resorption induced by strontium ranelate have beneficial effects on bone mass and bone quality (i.e., microarchitecture, geometry and intrinsic properties), resulting in a positive bone balance.

Although the role of strontium ranelate in related skeletal diseases is yet unknown, the effect of the properties of this drug on bone metabolism supports a potentially important role of promoting subchondral bone tissue homeostasis in OA patients. A phase III clinical study is presently underway to test the potential joint-structure-modifying effect of this drug in knee OA patients.

Calcitonin

Additional bone-active agents studied include salmon calcitonin [143]. Calcitonin is a well-known bone anti-resorptive agent that has demonstrated its efficacy in the treatment of osteoporosis and Paget's disease. Salmon calcitonin was administered daily for 1–8 weeks or from weeks 8 to 16 in the cruciate deficiency rabbit model [143]. The calcitonin-treated groups demonstrated smoother cartilage with minimal or no ulcerations, smaller osteophytes and hypercellularity where mild OA was present, suggesting regeneration. The investigators suggested that calcitonin worked in both prophylactic and therapeutic stages of OA. Studies in the ACL dog model [144,145] also showed that subcutaneous injections of calcitonin under therapeutic conditions reduced the progression of OA cartilage and subchondral bone changes as well as the level of serum markers of bone resorption up to 4 months after the surgery. A small phase II trial assessing the efficacy of oral salmon calcitonin in knee OA was recently published [146]. Results showed an improvement in function scores as well as a reduction in some biomarker levels. While this study was not analysed as an intention to treat and the sample size was not sufficiently powered, its promising results will perhaps lead to further larger trials.

Cathepsin K inhibitors

Cathepsin K is the most abundant cysteine protease expressed in the osteoclast, and it has been shown to play a critical role in the degradation of type I collagen containing bone matrix. This protease has also been demonstrated to be involved in OA cartilage degradation and subchondral bone alterations [8,147]. Transgenic mouse models have provided evidence supporting its important role in arthritis. Interestingly, cathepsin K is one of the few non-collagenase enzymes capable of degrading native fibrillar collagen types I and II [148]. Owing to the lack of adequate cross reactivity between the rat and human isoenzymes, animal models using cathepsin K inhibitors have been developed mostly in monkeys, and data have shown that inhibiting cathepsin K results in the prevention of bone loss. Majority of the clinical trials were phase II and almost all investigated the effect on BMD. To the authors' knowledge, one clinical phase II study was started in OA patients. Those clinical trials were stopped due to adverse events, which included skin changes. Inhibition of cathepsin K could be an

interesting candidate for OA as it could act on both cartilage and subchondral bone remodelling, provided that no safety issues arise in phase II and phase III studies.

Oestrogen

The effects of oestrogen on tissues such as bone have been extensively studied, and the pleiotropic effects of the female sex hormone are well established. Cartilage is not generally viewed as an oestrogen-responsive tissue. However, several epidemiological studies support the hypothesis that oestrogen may play a role in OA. Accordingly, the issue of chondroprotective properties of oestrogen has recently received increased attention. Treatment with oestrogen and a selective oestrogen-receptor modulator (SERM) was tested in ovariectomised rats and in postmenopausal women. A recent review analysing the effect of oestrogen treatment on animal models of OA revealed that the effect was inconclusive in 11 out of 22 studies reporting a beneficial effect on cartilage, whereas six studies in which SERMs were administered after ovariectomy described protective effects [149]. In a recent clinical trial conducted for 2 years in postmenopausal women, Karsdal et al. [150] investigated if the administration of a synthetic steroid with oestrogenic, androgenic and progestogenic properties would have similar dual actions on both bone and cartilage turnover. Data from this trial suggest that bone resorption can be attenuated, however, without the positive effects on cartilage degradation. These findings indicate an uncoupling effect on bone and cartilage from the synthetic steroid, which could be due to the pharmacology and biological activity of the product on these tissues.

It is suggested that currently available hormone replacement therapy and SERMs in clinical practice should not be recommended as therapy for OA [151]. However, there is hope that new treatment options based on compounds acting through the oestrogen receptor could be efficacious.

Vitamin D

It is well known that there is a decline in the circulating level of vitamin D with ageing, which may contribute to a stimulation of bone remodelling. A vitamin D deficiency in adults can result in increased bone turnover, enhanced bone loss and increased risk of fragility fracture. Although vitamin D was also shown to be an important hormonal contributor to cartilage and chondrocyte homeostasis and vitamin D receptors are present in chondrocytes [152], its role in OA is far less understood and still controversial. Indeed, while some clinical studies reported that vitamin D deficiency was associated with an increased risk of progression of knee OA and incidence of hip OA [153,154], others demonstrated no association between serum vitamin D levels and joint space loss or worsening cartilage score in knee OA [155,156]. Most of these studies used radiographic assessment of OA and one also employed MRI. However, in the study using MRI [155], the authors did not assess the whole knee or cartilage volume. Interestingly, in a recent study [157] in which the assessment of knee OA structural changes was performed both radiographically and by quantitative MRI, a change in serum vitamin D level was positively associated with change in cartilage volume. Despite the fact that the mechanisms of the action of vitamin D on cartilage remain unclear, one may speculate that it may have a direct effect on cartilage through vitamin-D-specific receptors. Conversely, but not exclusive of the latter, an effect on the subchondral bone metabolism is also possible as the vitamin D levels in serum were also associated with reduced subchondral bone area [157], a known risk factors for knee OA.

To our knowledge, there is presently no clinical trial addressing the effect of vitamin D on joint structure changes in human OA. However, there is an ongoing phase IV clinical trial, conducted in Europe and the USA, in knee OA patients undergoing unilateral total knee replacement, evaluating the effect of two doses of vitamin D on the pain as well as rehabilitation.

Other therapeutics

Medications commonly prescribed for OA include analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and viscosupplementation. However, patients are sometimes given complementary therapy. These complementary therapies include diacerein as well as nutraceutical agents such as the avocado/soybean unsaponifiables (ASU), glucosamine and CS. These products are prescribed in many countries worldwide. Some have undergone clinical trials and were shown to be effective on OA symptoms and sometimes also on joint structure changes. Clinical data supportive of diacerein as a modifying agent were shown in human hip OA [158]. On human OA knee subchondral

bone osteoblasts, data showed that diacerein reduces osteocalcin, urokinase, IL-6- and IL-1 β -induced MMP-13 production, as well as the synthesis of cathepsin K and MMP-13 on osteoclasts and the formation of these cells, factors that would contribute to curbing bone formation/resorption [159,160]. However, these *in vitro* findings need to be confirmed *in vivo*.

The disease-modifying effects of ASU were first shown in animal models. In the context of acting on the remodelling of OA subchondral bone, ASU treatment in the ACL dog model was shown to significantly improve the subchondral bone morphometry and calcified cartilage thickness as well as to reduce the severity of macroscopic and histological cartilage lesions, and the level of inducible nitric oxide synthase and MMP-13 [161]. The efficacy of ASU evaluated in the experimentally induced OA horse model showed that it had no effect on signs, pain or lameness, but there was a reduction in severity of articular cartilage erosion and synovial membrane inflammation and an increase in cartilage glycosaminoglycan synthesis [162]. Similarly, a positive effect of ASU was found on articular cartilage and subchondral bone pathophysiology in a sheep meniscectomy model of OA [163]. In humans, ASU showed significant symptomatic efficacy in the treatment of OA [164]. Furthermore, one study evaluating the effect of ASU in hip OA patients over 2 years demonstrated a significant reduction in joint space loss in the most severe cases [165]. With regard to glucosamine, the hydrochloride formula administered to the cruciate transection rabbit OA model reduced the high subchondral trabecular bone turnover [166]. A recent *in vitro* study carried out on pro-resorptive human OA subchondral bone osteoblasts demonstrated that the combination of glucosamine and CS could modulate the OPG/RANKL ratio in favour of reduced bone resorption [167]. These drugs have also been evaluated in many studies as agents to relieve pain, improve functional ability and slow disease progression, especially in knee OA patients [168,169].

Conclusion regarding bone-targeting drugs

The use of drugs/agents to treat knee OA and prevent the progression of structural changes needs further exploration. There is a good rationale behind the use of such agents since some are already being given to patients with osteolytic diseases and are proven safe for long-term administration. Some results from studies on humans affected with OA, however, have been disappointing. An explanation could be that the patients were in later stages of the disease upon study entry and that the technologies used to determine the disease-modifying effect were not sensitive enough. As data show that subchondral bone resorption is involved early during the OA process, further trials, perhaps in patients with less advanced disease, and with the use of more reliable and sensitive imaging technology, such as MRI and biomarkers, are needed.

Conclusion

Findings with regard to the physiological and pathological mechanisms of OA will make it possible to better target therapeutic approaches that could lead to the development of treatments to reduce or stop the progression of the disease. In that regard, this article summarises some of the knowledge we have today on the involvement of the subchondral bone in OA. It was long believed that the focal characteristic pathological feature of OA was the destruction of articular cartilage. Consequently, it is not surprising that investigations have concentrated for the past few decades on the mechanisms involved in cartilage degradation. In this article, substantial evidence is provided of the integral role in the OA process played by the changes in the metabolism of the subchondral bone.

The question that remains is whether subchondral bone alterations are the cause or a consequence of cartilage degeneration. However, as studies have demonstrated that the subchondral bone is the site of a number of active morphological changes at an early stage of the disease and that cross-talk with cartilage could very well occur, it is becoming increasingly evident that subchondral bone changes in OA are not merely secondary manifestations of the disease but are part of a more active component of OA. Not only is subchondral bone matrix altered in OA but also factors produced by OA subchondral bone cells can influence cartilage metabolism. This could possibly explain why increased subchondral bone activity can predict cartilage loss.

The ultimate goal in the treatment of OA is to improve or preserve the patient's joint structure by preventing its destruction. This changed view of the pathogenesis of OA from the involvement of the cartilage to the entire joint, and the recognition of early changes in the subchondral bone, provides the potential for new approaches to treatment of this disease. Targeting subchondral bone resorption and/or mineralisation thus represents an interesting approach to therapy for this disease, and data evaluating the drugs targeting bone remodelling raise the hope that new treatment options for OA may become available. Consequently, the field is opening up to a new era in which drugs and agents that can specifically block important mechanisms responsible for the joint tissue structural changes of OA can be brought into clinical trials.

Practice points

- I- Subchondral bone remodelling in OA
 - Alterations in subchondral bone activity occur early in the OA process
 - Early subchondral bone activity is resorptive
 - Although subchondral bone sclerosis is considered a hallmark of OA, it appears to occur at a later stage of the disease
 - Subchondral bone sclerosis is due to an increase in material density, and not mineral density
- II- Cross-talk between subchondral bone and cartilage
 - There is strong evidence of a diffusion of molecules/factors from the subchondral bone to the articular cartilage
 - This diffusion possibly occurs through the clefts and channels in the tidemark seen early in OA as well as through microcracks in the articular cartilage
- III- Markers of OA
 - Individual biomarkers are not very informative on their own of the disease process or of early disease
 - Clusters of markers are suggested to better correlate with disease progression and also could identify early disease
 - Imaging technology, such as MRI, is useful for assessing not only disease progression but also early events

Research agenda

Therapeutic agents targeting bone remodelling

- The promise of agents targeting bone remodelling in the treatment of OA has created an urgent need for clinical trials in humans
- With the recognition of early changes in subchondral bone during the OA process, patients with less advanced disease could be enrolled in standardised clinical trials instead of those at later stages

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