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Review

Bone–cartilage interface crosstalk in osteoarthritis: potential pathways and future therapeutic strategies

X.L. Yuan, H.Y. Meng, Y.C. Wang, J. Peng, Q.Y. Guo, A.Y. Wang*, S.B. Lu

Institute of Orthopedics, Chinese PLA General Hospital, Fuxing 28# Road, Beijing, China

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SUMMARY

Currently, osteoarthritis (OA) is considered a disease of the entire joint, which is not simply a process of wear and tear but rather abnormal remodelling and joint failure of an organ. The bone–cartilage interface is therefore a functioning synergistic unit, with a close physical association between subchondral bone and cartilage suggesting the existence of biochemical and molecular crosstalk across the OA interface. The crosstalk at the bone–cartilage interface may be elevated in OA *in vivo* and *in vitro*. Increased vascularisation and formation of microcracks associated with abnormal bone remodelling in joints during OA facilitate molecular transport from cartilage to bone and *vice versa*. Recent reports suggest that several critical signalling pathways and biological factors are key regulators and activate cellular and molecular processes in crosstalk among joint compartments. Therapeutic interventions including angiogenesis inhibitors, agonists/antagonists of molecules and drugs targeting bone remodelling are potential candidates for this interaction. This review summarised the premise for the presence of crosstalk in bone–cartilage interface as well as the current knowledge of the major signalling pathways and molecular interactions that regulate OA progression. A better understanding of crosstalk in bone–cartilage interface may lead to development of more effective strategies for treating OA patients.

Introduction

Osteoarthritis (OA) represents a group of degenerative joint diseases characterised by degeneration of articular cartilage, synovial inflammation and changes in periarticular and subchondral bone. OA is considered a disease of the entire joint, involving all joint tissues[1]. Cartilage, synovium, bone and bone marrow as well as menisci, ligaments, muscles and neural tissues are involved in the complex initiation and progression of the disease. OA is not simply a process of wear and tear but rather an abnormal remodelling and joint failure[2].

The bone–cartilage interface contains the region between the deep layers of articular cartilage and the underlying subchondral bone. This region comprises the tidemark, calcified cartilage layer and subchondral bone plate. The bone–cartilage interface is therefore a complex functional unit and biocomposite at the centre of joint function and disease in which the individual components interact cooperatively and synergistically[3]. Biomechanical and biological processes result in alterations in the composition, structure and functional properties of this unit. Given the intimate contact between bone and cartilage, alterations of either tissue will modulate the properties and function of the other components, as shown in Fig. 1[4].

The close physical association between subchondral bone and cartilage suggests the possibility of biochemical and molecular crosstalk across the interface in healthy and osteoarthritic joints. The perspective that calcified cartilage and bone is an insurmountable and impermeable barrier to soluble molecules and functional interaction has changed, and new evidence indicates the important role played by the bone–cartilage biomechanical unit in the development and progression of OA. Increased vascular communication channels, fissures and microcracks through the complex and the irregular anatomy of the bone–cartilage junction—which could act as a transport conduit—facilitate molecular transport, suggesting that crosstalk in molecular interactions exists between cartilage and the subchondral bone. Mediators produced from both tissues may pass from one zone to another, affecting the homeostasis of neighbouring tissues. Collectively, this evidence indicates that crosstalk in the bone–cartilage interface is a holistic system, highlighting the involvement of multiple factors and their contribution to OA mechanisms. A comprehensive approach could determine the cascade of events and integrate molecular and cellular data regarding the progression of OA.

* Address correspondence and reprint requests to: A.Y. Wang, Institute of Orthopedics, Chinese PLA General Hospital, Fuxing 28# Road, Beijing, China.
E-mail address: aiyuanwang301@126.com (A.Y. Wang).

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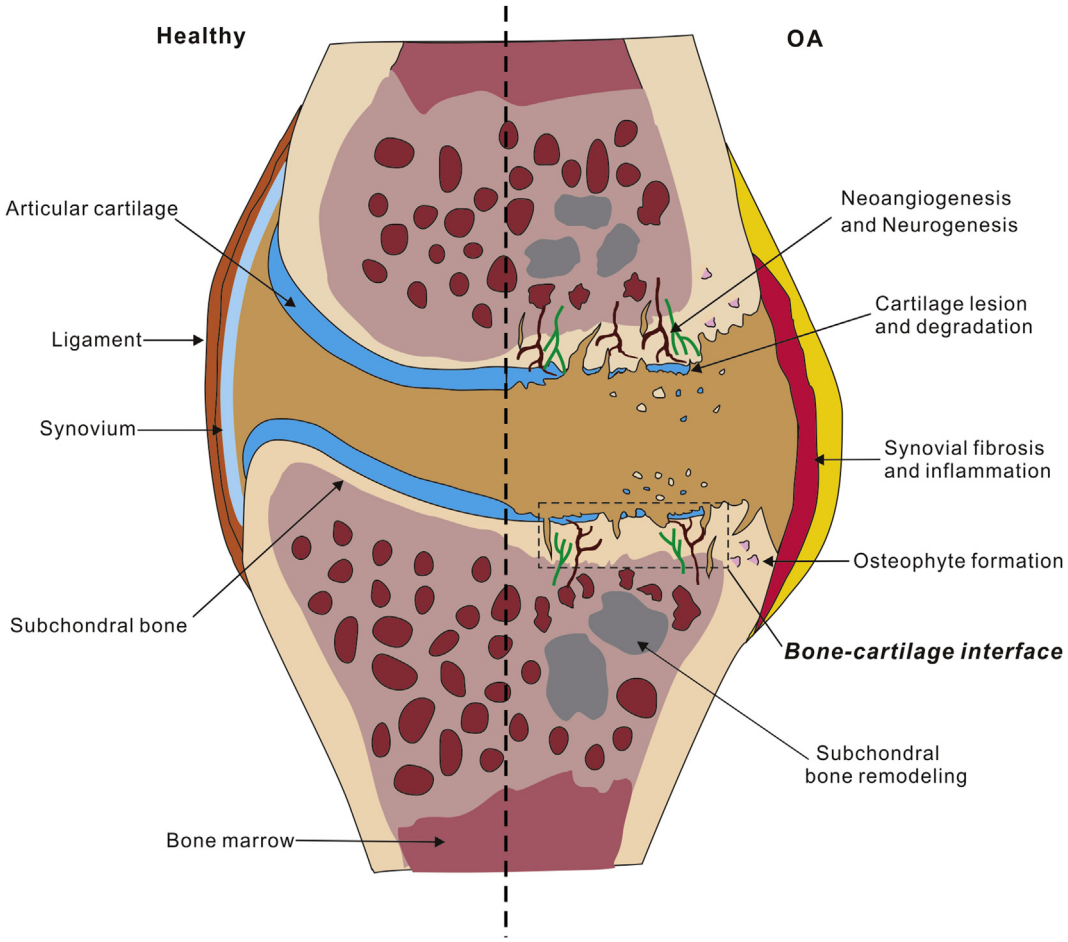

Fig. 1. Differences between normal and osteoarthritic joints. OA is considered to involve all joint tissues. The bone–cartilage interface is at the centre of joint function and disease.

In this review, we summarise the biological and pathological evidence for the presence of crosstalk in the bone–cartilage interface. In particular, we discussed the critical signalling pathways and molecular interactions *in vivo* and *in vitro*. We also focused on therapeutic interventions that modulate crosstalk and improve OA outcomes.

The basis for elevated crosstalk in the osteoarthritic bone–cartilage interface

Articular cartilage and subchondral bone alterations

Osteochondral changes include loss of cartilage, increased subchondral bone thickness, decreased subchondral trabecular bone mass, new osteophyte formation at the marginal joint, gradual development of bone cysts and bone marrow lesions (BML). Other changes include tidemark duplication, thickening of the calcified cartilage layer, cracks extending through the cartilage down to the subchondral bone, and the development of chondrocyte clones, connective vascular structures and blood vessels[5] (Fig. 2).

During the progressive stages of OA, degeneration in the material properties and structural integrity of articular cartilage can be caused by associated changes in the molecular composition of the matrix. Articular chondrocytes increase the synthesis of matrix molecules but also contribute to their own destruction by synthesising proinflammatory cytokines, including interleukin (IL)1, and tissue-destructive enzymes such as matrix metalloproteinases (MMPs) and metalloproteinases with thrombospondin motifs (ADAMTS)[6]. Extensive cellular changes are accompanied by increased expression of molecules related to chondrocyte hypertrophy and terminal differentiation, such as vascular endothelial growth factor (VEGF), runt-related transcription factor 2 (RUNX2) and MMP13[7]. The normally quiescent chondrocytes undergo a phenotypic shift to become activated cells. Chondrocyte proliferation, cluster formation and increased production of matrix proteins and enzymes that degrade specific ECM proteins are followed by amplified catabolic activity leading to matrix remodelling and loss of the cartilaginous structure, inappropriate hypertrophy-like maturation and cartilage calcification, fissures and microcracks. As a result, the cartilage surface becomes rough, accompanied by fibrillation and a reduction in thickness[8,9].

Subchondral bone remodelling is the initial step in abnormal mechanical loading and involves bone matrix formation and degradation by osteoblasts and osteoclasts. Bone turnover is likely to play a pivotal role in the pathogenesis of OA[10]. OA is associated with early bone loss due to increased bone remodelling, followed by slow turnover leading to densification of the subchondral plate and complete loss of cartilage[11]. Elevated bone remodelling and its associated stimulated vascularity are reportedly indispensable for the progression of OA[11].

In the early stages of OA, bone remodelling and subchondral bone loss are elevated. An increased number of osteoclasts with reduced trabecular thickness and increased bone loss as well as lower modulus were detected. The increased rates of bone remodelling

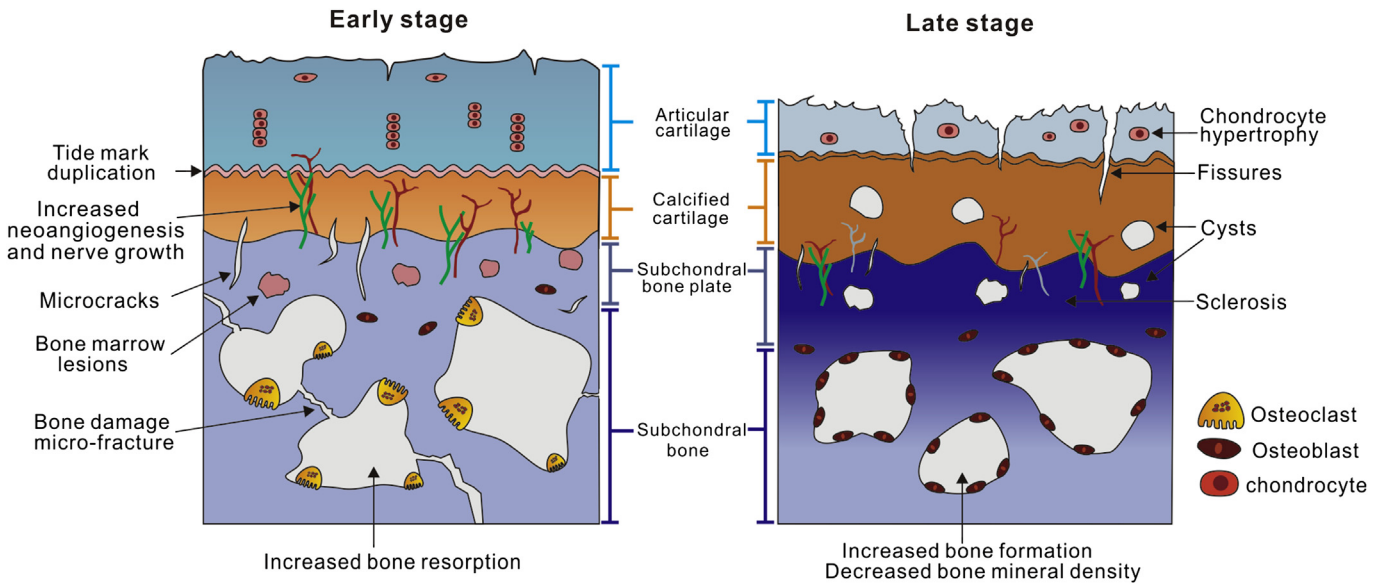

Fig. 2. The basis of crosstalk in early and late stages of OA. In the early stages of OA, mechanical overload promotes subchondral bone resorption and cartilage degradation. Neoangiogenesis and neurogenesis pass the tidemark into the cartilage. Microcracks and BMLs occur secondary to abnormal mechanical load. In the late stages of OA, cartilage matrix loss and chondrocyte hypertrophy, followed by the appearance of fissures and the cutting of channels, extend down the subchondral plate. In contrast, the thickened calcified cartilage contains apoptotic chondrocytes and cysts. Subchondral bone sclerosis is related to an increase in osteoblastic bone formation.

may cause alterations in joint shape and load transmission that predispose to progressive cartilage loss. Microstructural impairment of subchondral bone was associated with increased remodelling of aggravated cartilage damage in OA rabbits with osteoporosis[12]. Thinning and increased porosity of the subchondral bone plate were significantly related to cartilage damage[13]. In contrast, subchondral bone becomes sclerotic in late OA. Bone formation and osteoblastic activity dominate in this phase a higher bone density and volume[14]. Although late-stage OA is associated with thickening of subchondral bone, the stiffness of subchondral bone is low, accompanied by decreased mineralisation[15].

Bone attrition, defined as depression or flattening of the subchondral bony surface unrelated to gross fracture[16], is the decrease or loss of bone height and contour that accompanies the osteoarthritic changes in bone. These changes likely represent remodelling of subchondral bone which can occur in the early and middle stages of OA. Subchondral bone attrition is also associated with the severity of cartilage loss in adjacent regions, and areas of BML detected by MRI, indicating an increased load on the bone–cartilage biomechanical unit[17,18].

BMLs are indicators of OA progression and are considered an important risk factor for structural deterioration[19]. These changes are consistent with localised activation of bone repair processes accompanying bone remodelling to increase the mechanical load[20]. The localisation of BMLs was associated with areas of severe cartilage loss in the overlying articular surface and revealed fat necrosis and localised marrow fibrosis associated with microfractures of the trabecular bone[21]. Bone marrow oedema and subchondral cysts are useful for assessing OA progression by MRI. BMLs appear sclerotic due to increased bone volume fraction and decreased tissue mineral density as well as increased trabecular thickness. These changes may render this area mechanically compromised and thus susceptible to attrition[22].

Osteochondral angiogenesis and nerve growth

Angiogenesis and sensory nerve growth are closely integrated processes that are potentially linked to the elevated crosstalk in OA. In OA, increased osteoclast activity causes channels to extend from the subchondral bone and ultimately pass across the tidemark into the articular cartilage. Channel formation in regions of vascular invasion is associated with localised subchondral bone marrow replacement by VEGF-expressing fibrovascular tissue and infiltration of inflammatory cells into the marrow spaces, with increased endothelial cell proliferation and vascular density[23]. The increased number of blood vessels is accompanied by extensions of sympathetic and sensory nerves from the subchondral bone to occupy the osteochondral channels and invade the normal cartilage to increase nerve growth factor expression within vascular channels[24]. Animal models have shown increased angiogenic activity of subchondral bone in the early to progressive stages of OA, followed by vascular invasion into the osteochondral junction[25,26].

Crosstalk in bone–cartilage interface

Studies using sodium fluorescein[27] have demonstrated *in vivo* that a number of molecules can diffuse through the bone–cartilage interface. Variations in the degree of mineralisation, together with the microcracks and fissures that occur in OA, likely further facilitate this transport and cellular crosstalk. Hwang *et al.*[28] reported that the hydraulic conductance of the articular cartilage and subchondral bone plate increases with OA progression. These findings are linked to the increased density of enlarged subchondral canals penetrating the calcified cartilage in samples with partially eroded cartilage.

During the progression of OA, an increasing number of large transport conduits (vascular channels or marrow cavities) break the osteochondral interface and increase significantly the overall transport capacity across the joint, overcoming the barrier posed by the denser ECM. Pan *et al.* found using a photobleaching (FLIP) method that sodium fluorescein diffuses across the calcified cartilage and osteochondral interface at a much higher rate in two murine models of OA than in normal joints; both cartilage damage and vascular invasion can increase crosstalk *via* diffusion of small molecules[29]. Bone turnover also leads to elevated crosstalk between cartilage and subchondral bone during OA. A study using OA

mouse models showed early and temporal subchondral plate porosity and increased perforation in OA with enhanced biochemical and mechanical interactions among the subchondral trabeculae, bone marrow cells and articular cartilage[30].

Cellular interactions in crosstalk *in vitro*

Crosstalk in molecular interactions between chondrocytes and osteoblasts, osteoclasts and osteocytes has been investigated using a number of *in vitro* systems. These cellular interaction experiments (Table I) suggest potential mechanisms of action in an artificial environment and could provide a basis for further *in vivo* models. Sanchez *et al.* showed that subchondral bone osteoblasts from OA patients exhibit increased production of bone anabolic molecules and altered phenotypes[31]. Furthermore, Westacott *et al.* suggested that various cytokines and growth factors secreted by subchondral bone cells of OA patients promote loss of cartilage proteoglycans[32].

Local factors secreted by osteoblasts also contribute to cartilage degradation. Osteoblasts derived from osteoarthritic sclerotic bone with osteoarthritic articular chondrocytes exhibit decreased production of aggrecan and chondrocyte markers (SOX9 and COL2), but increased production of MMP3 and MMP13, suggesting that local factors secreted by osteoblasts initiate chondrocyte hypertrophy and matrix mineralisation[33,34].

Recently, possible crosstalk between osteoclasts or macrophages and chondrocytes has been reported. Co-culture of human articular chondrocytes and macrophages has revealed crosstalk involving MMP-1, MMP-3 and MMP-9[35]. MMP-9 in co-culture medium originated from macrophages and its activation required not only chondrocyte-derived factors but also the presence of MMP-3 and MMP-1; however, the mechanism involved remains unclear. Additionally, similar crosstalk occurs between osteoblasts and osteocytes. Extracellular matrix produced by OA subchondral bone osteoblasts results in downregulation of integrin β1 expression and upregulation of MMP2 and MMP9 expression, suggesting that osteoblasts may lead to abnormal osteocyte phenotypic changes[36].

In vivo molecular interactions in crosstalk processes (Fig. 3)

Molecular interactions in the bone–cartilage interface provide insight into the specific mechanisms underlying the effects of crucial biological factors and signalling pathways on cartilage-bone pathophysiology in OA (Table I). Factors released from subchondral bone tissue may induce OA cartilage degradation. Conversely, subchondral bone remodelling may be a result of factors secreted from cartilage. An imbalance of molecular interactions may also lead to cartilage damage and subchondral bone remodelling, contributing to abnormal crosstalk[37].

Biological factors

Osteoprotegerin (OPG), receptor activator of nuclear factor-κB (RANK) and RANK ligand (RANKL)

The molecular triad of OPG, RANK and RANKL is a key cytokine system involved in the regulation of bone resorption[38]. In the bone remodelling process, RANKL, expressed by osteoblastic lineage cells as well as chondrocytes, is essential for mediating bone resorption through osteoclastogenesis and activating mature osteoclasts by binding to RANK, which is present on precursor and mature osteoclasts and expressed only by chondrocytes. OPG is produced by osteoblasts/stromal cells and inhibits the binding of RANKL to RANK[39,40].

The RANKL to OPG ratio was reported to be increased in human OA cartilage compared with normal cartilage, as well as in rabbit OA subchondral bone[12,38]. In recent research involving human subjects, this ratio was increased in early-stage OA but decreased in late-stage OA[41]. Inhibition of RANKL by systemic[42] or intra-articular[43] administration of OPG in mice prevented bone and cartilage degradation, and treatment with a modified OPG (OPG-Fc) attenuated the OA changes and reduced pain behaviour in rats[44].

Hepatocyte growth factor (HGF)

Another molecular interaction across the bone–cartilage interface involves HGF, which is localised in osteoarthritic calcified cartilage and the deep zone of mild osteoarthritic cartilage. HGF can promote synthesis of type II collagen and proteoglycan as well as collagenase-3 and MMP3 production[45]. However, research on human OA showed that HGF is synthesised by osteoarthritic osteoblasts from the subchondral bone plate and diffuses to the cartilage region, suggesting that subchondral bone osteoblasts are responsible for the HGF present in OA cartilage[46]. In addition, HGF is a potent inhibitor of bone resorption but increases osteoblastic function[47], which may induce a positive bone remodelling balance by both decreasing bone resorption and increasing bone formation.

Hypoxia-inducible factor-2α (HIF-2α)

HIF-2α, which is encoded by *Epas1*, is expressed mainly in highly differentiated chondrocytes and is a major catabolic transcription factor in the OA process. Another possible example of crosstalk at the bone–cartilage interface is based on the increased HIF-2α expression in osteoarthritic cartilage, which can lead to cartilage breakdown and endochondral bone formation[48].

Yang *et al.* first showed that HIF-2α causes progressive cartilage damage by upregulating directly the expression of a set of matrix-degradative enzymes. Moreover, mice transgenic for Epas1 only in chondrocytes showed spontaneous cartilage destruction, whereas heterozygous genetic deletion of Epas1 suppressed cartilage destruction with concomitant reductions in catabolic factor levels[49]. Furthermore, increased HIF-2α levels in OA chondrocytes were associated with increased apoptosis of articular chondrocytes,

Table I
The main methods used for the inclusion of studies on crosstalk

Crosstalk	Methods	Observation index	Typical Reference
Cellular interaction	Cell Co-culture	Various cytokines, growth factors secreted by osteoblast, osteoclast and chondrocyte	Sanchez *et al.*[33]
Molecular interaction	Photobleaching (FLIP)	The diffusivity of sodium fluorescein	Pan *et al.*[29]
	Perfusion testing	Hydraulic conductance	Hwang *et al.*[28]
	Inhibition of specific factors or signalling pathways	IGF, TGF-β, β-catenin	Zhu *et al.*[61]
	Activation of specific factors or signalling pathways	Wnt	Zhu *et al.*[62]
	Antagonists and agonists of signalling pathways	WISP-1, Wnt 16, Wnt2B	Blom *et al.*[66]
	Knockout of specific genes	Wnt/β-catenin, Epas1	Ryu *et al.*[50]
	Transgenic technology	Epas1	Yang *et al.*[49]

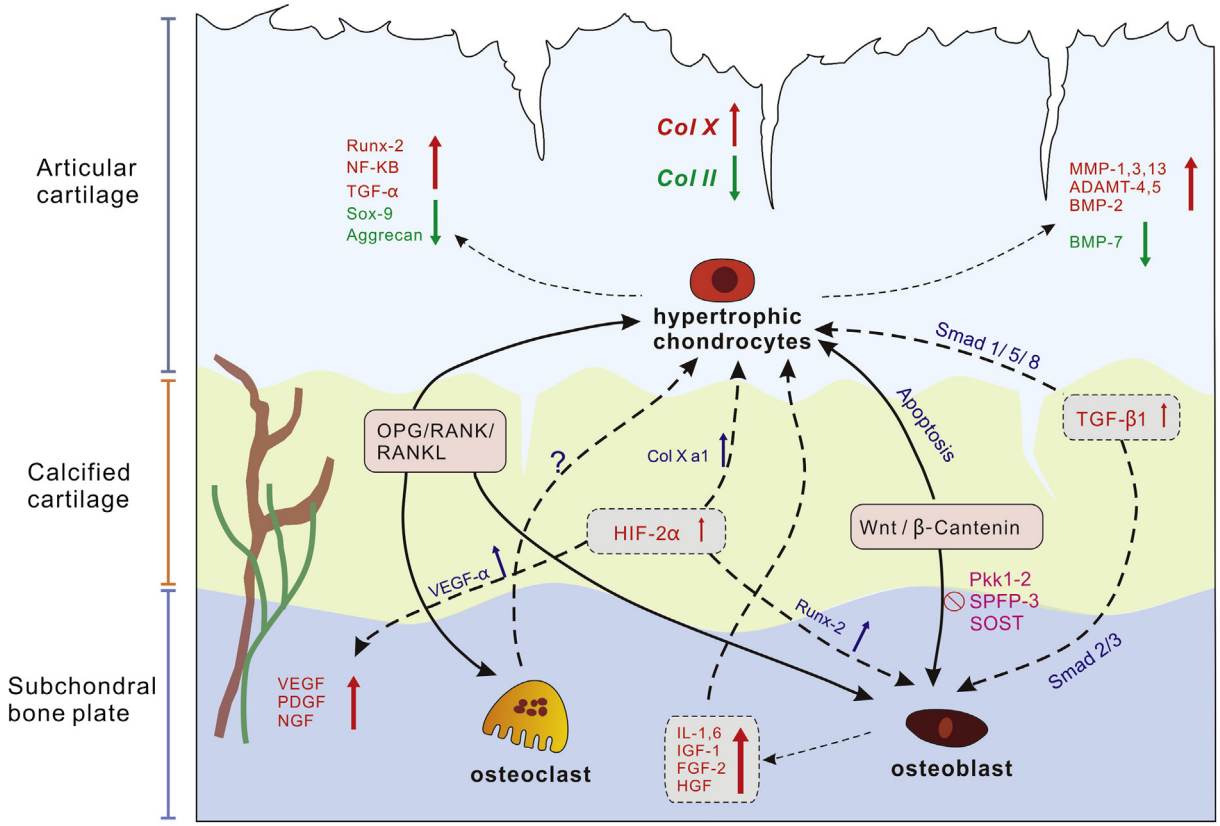

Fig. 3. Molecular and cellular crosstalk at the osteochondral junction in OA. The presence of connections (microfractures, fissures, vascular channels and blood vessels) between subchondral bone and cartilage contributes to elevated crosstalk between chondrocytes, osteoblasts and osteoclasts through biological factors and signalling pathways. Subchondral bone is exposed to factors produced by hypertrophic chondrocytes, such as MMPs and ADAMTs. Reciprocally, invasion of the articular cartilage by vascular channels exposes chondrocytes to cytokines and growth factors from subchondral tissues, such as VEGF, NGF, IL-1, IL-6, HGF, or IGF-1. The imbalance of OPG/RANKL has multiple effects on bone and cartilage. HIF-2α is associated with advancing endochondral ossification and chondrocyte hypertrophy. TGF-β1 and The Wnt/β-catenin signalling pathways have emerged as key regulators of bone and cartilage. The effects of specific Wnt agonists and antagonists—including sFRP-3 and DKK1-2—on these tissues remain to be elucidated.

enhanced Fas expression and cartilage destruction. Chondrocyte-specific knockout of Epas1 and Fas deficiency in mice suppressed chondrocyte apoptosis and inhibited OA cartilage destruction[50].

Moreover, elevated HIF-2α may contribute to endochondral ossification in OA[51]. HIF-2α is essential for chondrocyte hypertrophic differentiation, followed by cartilage matrix degradation and vascular invasion, and enhances the promoter activities of many key factors, including COL10A1, MMP13, VEGFA, RUNX2 and Indian hedgehog (IHH). The signals that induce endochondral ossification cause cartilage degradation at the centre of the joint and osteophyte formation at the periphery[52].

HIF-2α expression is related to OA development; expression is induced and peaks in early-stage OA but is downregulated in late-stage OA. HIF-2α may suppress chondrocyte autophagy and counteract HIF-1α activity[53]. Consequently, the control of cartilage homeostasis may be related to the balance between HIF-1α and HIF-2α activities because HIF-1α functions to maintain cartilage while HIF-2α induces endochondral ossification and cartilage degradation. The shift from HIF-1α to HIF-2α expression might be a pathogenetic feature of OA and has been considered a potential target for a novel therapeutic strategy[54].

Bone–cartilage modulatory pathway

Transforming growth factor β (TGF-β)

TGF-β signalling is required for maintenance of the metabolic homeostasis and structural integrity of healthy cartilage. TGF-β is highly expressed in normal cartilage but almost absent in osteoarthritic cartilage. Loss or interruption of TGF-β signalling in cartilage results in loss of proteoglycans and cartilage degeneration[55]. Inhibition of endogenous TGF-β leads to increased damage to cartilage[56] and prevents osteophyte formation and synovial fibrosis[57].

The effect of TGF-β signalling on cartilage includes promotion of homeostasis through the classical Smad2/3 pathway *via* actin ALK5 and induction of chondrocyte hypertrophy through the Smad1/5/8 pathway *via* ALK1[58]. Increased production of TGF-β by deteriorating cartilage under osteoarthritic conditions together with an elevated ALK1 to ALK5 ratio might affect homeostasis of both the cartilage and bone in joints, indicating an intermediate crosstalk between cartilage and bone. Zhen *et al.* showed that TGF-β is activated in subchondral bone in response to altered mechanical loading in an ACLT mouse model of OA[59]. Increased TGF-β levels in subchondral bone increase the numbers of mesenchymal stem cells (MSCs), osteoprogenitors and osteoblasts, leading to aberrant bone remodelling and angiogenesis. Whether MSC-specific genetic ablation of TGF-β receptor II (Tgfbr2) or antibody-mediated neutralisation of TGF-β in subchondral bone can reduce osteoarthritic damage and improve bone parameters, cartilage structure and joint functionality, remains unclear.

Wnt/β-catenin pathway

Studies have shown that the Wnt/β-catenin signalling pathway plays a critical modulatory role in maintaining the bone-cartilage biochemical unit. The biochemical effects of activated Wnt signalling contribute to excessive bone remodelling and degradation of

Table II
Summary of main clinical studies using inhibiting crosstalk agents for OA treatment

Category	Therapeutic agents	Study design	Case	Time	Treatment dose/procedure	OA location	Outcomes	Study authors
Anti-NGF	Tanezumab	Proof-of-concept study,	365	6 m	10,25,50,100,200 μg/kg on day 1 and 56 IV	Knee	Improvement in WOMAC score, knee pain and PGA measure	Lane *et al.*(2010)[70]
		Phase II, multicenter, open-label, multiple-dose extension of an earlier randomized trial	239	56 w	50 μg/kg on Days 1 and 56 with subsequent doses administered at 8-week intervals IV	Knee	Modified WOMAC physical function score and SGA with a low incidence of AEs	Schnitzer *et al.*(2011)[71] NCT00399490
		Randomized, double-blind, placebo-controlled, dose-escalation, single intravenous (IV) dose, two-part study	67	13 w/17 w	Part I:10,25,50,100,200 μg/kg Part II: 10,25,50,100 μg/kg IV	Knee	Improved index knee pain and WOMAC pain, physical function, stiffness subscales	Nagashima *et al.* (2011) NCT00669409
		Randomized, double-blind, placebo-controlled phase III trial	518	32 w	2.5,5,10 mg IV	Knee	significant improvement in WOMAC Pain, Physical Function subscale and PGA score	Brown *et al.*(2012)[72] NCT00733902
		Phase III, placebo- and active-controlled study	311	18 W	5,10 mg in 8 w intervals IV	Hip/Knee	Improved WOMAC pain, physical function, stiffness subscales and PGA	Spierings *et al.*(2013)[75]
		Phase III, randomized, double-blind, placebo-controlled, parallel-group, multicentre trial	352	32 w	2.5,5,10 mg at weeks 0, 8 and 16 IV combined with diclofenac 75 mg bid	Hip/Knee	Improved WOMAC Pain, Physical Function subscale and PGA score; Higher incidence of AEs and no new safety signals emerge	Balanescu *et al.*(2013)[73] NCT00864097
		Randomized, double-blind, placebo-controlled, phase III trial	466	32 W	2.5,5,10 mg at weeks 0, 8 and 16 IV	Hip	Modified WOMAC pain, physical function score and PGA	Brown *et al.*(2013)[74] NCT00744471
	Fulranumab	Randomized, double-blind, placebo-controlled, and dose-ranging study	390	26 w	1,3 mg Q4wk; 3,8,10 Q8wk IH	Hip/Knee	Positive effect on OAPI,WOMAC subscales and score, BPI-SF subscales and PGA score; well tolerated	Sanga *et al.*(2013)[76] NCT00973141
Bisphosphonates	Alendronate	CS	57	36 m		Knee	Modified WOMAC pain scale, MRI findings	Carbone *et al.*(2004)[90]
		Prospective randomized case–control study	33	24 m	35 mg/week PO	Hip	Improved VAS and WOMAC pain scores, Decreased urinary NTX-I,CTX-II, No improvement of radiograph progression	Nishi *et al.*(2012)[91]
	Risedronate	Prospective, double-blind, placebo-controlled study (BRISK study)	151	12 m	5 mg/d or 15 mg/d PO	Knee	Improved joint space narrowing of X-ray, WOMAC index and PGA scores	Spector *et al.*(2005)[86]
		Two parallel phase III Prospective, double-blind, multi-centre, placebo-controlled study	1861	24 m	5 mg/d,15 mg/d,35 mg/wk(EU) PO 5 mg/d,15 mg/d,50 mg/wk(NA) PO	Knee	No improvement of WOMAC, PGA scores and radiograph progression, Decreased urinary NTX-I,CTX-II	Bingham *et al.*(2006)[88] Garnero *et al.*(2008)[89]
		Double blind, multi-centre, placebo-controlled study (KOSTAR study)	929	24 m	5 mg/d,15 mg/d,50 mg/wk PO	Knee	Modified X-ray and FSA	Buckland-Wright *et al.*(2007)[87]
		Randomized non-blinded case–control study	33	18 m	2.5 mg/d PO	Knee	No symptomatic benefit	Kawasaki *et al.*(2008)
	Clodronate	PhaseII randomized partially blind clinical trial	117	1 m	0.5/1/2 mg 1× IA/week for 4 weeks, 1 mg 2× IA/week for 2 weeks	Knee	Improved VAS scores, Lequesne index, Joint extension and mobility scores	Rossini *et al.* (2009)[92]
	Zoledronic acid	Randomized double-blind placebo-controlled trial	31	12 m	5 mg/100 ml IV	Knee	Decreased VAS scores and BML area, No improvement in KOOS scale	Laslett *et al.* (2012)[93] ACTRN 12609000399291
Calcitonin	Salmon calcitonin	Phase II Randomized double blind, placebo-controlled trial	152	3 m	0.15 mg/d,0.4 mg/d,1 mg/d,2.5 mg/d PO	Knee	Decreased urinary CTX-I,CTX-II	Bagger *et al.*(2005)[100]
		Randomized, double-blind trial	35	3 m	0.5 mg/d,1 mg/d PO	Knee	Improved Lequesne's algofunctional index scores, Decreased urinary and serum levels of CTX-II, C2C and MMP13	Manicourt *et al.*(2006)[101]
		Phase I randomized, double-blind, double-dummy, placebo-controlled, gender-stratified study	50	2 w	0.6 mg/bid,0.8 mg/bid PO	Knee	Reduced serum CTX-I and urinary CTX-II	Karsdal *et al.*(2010)[102] NCT00486369
		Self-control study	220	12 m	200 IU/d Nasal spray form	Knee	Improved VAS and WOMAC scores	Esenyel *et al.*(2012)[104]
		Randomized case–control study	30	6 m	200 IU/d nasal spray form	Knee	Improved VAS and WOMAC scores, Decreased serum NO and urinary CTX-II	Armagan *et al.*(2012)[103]

Strontium ranelate	*Post-hoc* analysis of pooled data from SOTI and TROPOS	566 36 m	2 g/d PO	Spine	Reduced radiographic progression and improvement of back pain	Bruyere *et al.*(2008)[109]	
	Randomized, double blind, placebo-controlled phase III TROPOS study	565 36 m	2 g/d PO	All kinds	Significantly decrease in urinary CTX-II over a 12-month period	Alexandersen *et al.*(2011)[110]	
	International, multicentre, randomized, double-blind, placebo-controlled phase III trial (SEKOIA)	1124 36 m	1 g/d,2 g/d PO	Knee	Reduction in WOMAC and VAS score, improvement of radiological progression in JSW, better effect in 2 g/d	Reginster *et al.*(2013)[111] ISRCTN41323372	

SGA: Subject Global Assessment; IV: intravenous injection; IH: hypodermic injection; IA: Intra-articular administration; OAPI: Osteoarthritis pain intensity; BPI-SF: Brief Pain Inventory–Short Form; BRISK: British study of risedronate in structure and symptoms of knee OA; FSA: fractal signature analysis; KOSTAR: Knee OA Structural Arthritis study; VAS: visual analogue score; PO: oral administration; SOTI: Spinal Osteoporosis Therapeutic Intervention; TROPOS: Treatment Of Peripheral Osteoporosis.

the cartilage matrix[60]. Activation or inhibition of β-catenin can result in cartilage damage, OA-like lesions and chondrocyte apoptosis, perturb the articular chondrocyte phenotype, and upregulate markers associated with hypertrophy and terminal differentiation[61,62]. Conversely, overexpression of Wnt signalling is deleterious to chondrocytes, leading to OA-like disease. Frizzled-related protein 3 (FRP3) is a Wnt antagonist. FRP-knockout mice are more susceptible to cartilage damage in OA and show MMP activation and loss of proteoglycans[63]. Besides chondrogenesis, Wnt signalling is essential for bone remodelling. Activation of Wnt signalling, either through knockdown of Wnt antagonists or by overexpression of β-catenin, resulted in increased bone formation in mice, leading to thicker and stiffer bones[62,63].

Various antagonists and agonists of the Wnt/β-catenin pathway including sclerostin (SOST), secreted frizzled-related protein (sFRP) and Dickkopf (DKK1) may be crosstalk effector molecules that function to maintain normal homeostasis. sFRP-3 might protect against the progression of cartilage loss in OA. An elevated serum Dkk-1 level is associated with reduced progression of hip OA[64]. Two rat models of OA treated with antisense oligonucleotide DKK1 showed less cartilage damage and reduced bone remodelling[65]. Moreover, increased expression of Wnt signalling agonists, such as Wnt-induced signalling protein 1 (WISP-1), Wnt16 and Wnt-2B, were reported in human OA cartilage and may contribute to cartilage degradation by upregulating MMPs and aggreganases[66].

Another example of Wnt/β-catenin pathway crosstalk with TGF-β signalling has been reported recently. The secretion of WISP-1 may have an osteogenic effect on the underlying subchondral bone by enhancing osteoblast differentiation. Additionally, WISP-1 can modulate TGF-β signalling by inhibiting Smad2[67]. The interaction between TGF-β and WNT pathways mediated by WISP-1 may play a critical role during OA progression at the bone–cartilage interface.

Therapeutic strategies based on crosstalk (Table II)

Targets of angiogenesis and neurogenesis: channel closing

Targeting and inhibiting angiogenesis and neurogenesis to decrease the molecular interaction and maintain the integrity of the osteochondral junction may therefore identify new therapeutic strategies for treating OA and yield direct symptomatic benefits[68]. Strategies include direct inhibition by agents that act on VEGF and β-NGF, or indirect inhibition of the matrix degradation and osteochondral channel formation necessary for the growth of blood vessels. The angiogenesis inhibitor PPI-2458 reduced the number of vascularised channels and joint damage while simultaneously reducing pain behaviour in an OA animal model[26]. Because the neovascularisation mechanisms are similar in tumours and osteoarthritic joints, the VEGF-blocking antibodies bevacizumab, ranibizumab and ramucirumab may prove beneficial for the treatment of OA. Intravenous administration of bevacizumab can enhance articular cartilage repair in an osteochondral defect model[69].

Dual inhibition of angiogenesis and nerve growth could be an attractive therapeutic strategy for patients with OA. The humanised monoclonal anti-NGF antibody tanezumab blocks NGF binding to its receptors, thereby improving function and reducing joint pain in patients with knee OA[70]. In a phase II clinical trial by Schnitzer *et al.*[71], long-term repeated injections of tanezumab in patients with moderate-to-severe knee OA provided continued pain relief and improved function with a low incidence of side effects. Moreover, tanezumab is well tolerated and effective for analgesic treatment of knee OA[72]. A 32-week follow-up clinical trial of sustained-release tanezumab combined with diclofenac in patients with knee or hip OA showed a higher incidence of adverse events (AEs), which

suggests that combination therapy is unfavourable and further investigations of tanezumab monotherapy are required[73]. In recent phase III studies, Brown *et al.* analysed 466 patients with osteoarthritic hips given three intravenous tanezumab injections at 8-week intervals. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain and physical function subscale scores demonstrated statistically significant improvements as compared with baseline or placebo[74]. Similar results were reported by another phase III clinical trial in osteoarthritic hip or knee patients[75]. Sanga *et al.* found that subcutaneous injections of the new human monoclonal anti-NGF antibody fulranumab resulted in significant pain reduction and improved physical function and was generally well tolerated[76].

Targets of subchondral bone remodelling: structural repair

Osteochondral structure provides ample pathways, including vascular tunnels, fissures and cracks, for intimate molecular crosstalk. The accelerated remodelling process in osteoarthritic subchondral bone may cause osteoclastic resorption and increased porosity in subchondral regions, leading to elevated crosstalk between bone and cartilage. Based on the results demonstrating a remodelling process in the subchondral bone during OA, whether factors capable of influencing bone remodelling could be used as therapeutic strategies remains questionable. Various antiresorptive agents appear well suited to preventing the progression of OA as modulators of subchondral bone remodelling.

Bisphosphonates (BPs)

Various BPs (alendronate, risedronate, tiludronate and zoledronic acid) decrease vascular invasion of calcified cartilage, inhibit subchondral bone remodelling, reduce subchondral bone loss and inhibit osteophyte formation[77,78]. These agents also modulate the OPG/RANKL system, inhibit osteoclast activity and reduce pain behaviour[79,80]. In the rat ACL transection model, Hayami *et al.* demonstrated dose-dependent chondroprotective effects of alendronate in terms of osteophyte formation inhibition and reductions in the levels of cartilage degradation biomarkers[78]. Other studies have confirmed the beneficial chondroprotective effects and bone remodelling of alendronate and pamidronate in experimental OA models[81,82]. However, different results were reported from use of the spontaneous guinea pig model, in which alendronate increased the bone density and mineral content and was associated with aggravated cartilage degradation[83]. Additionally, the treatment timepoint is crucial for the effectiveness of BPs. Zhu *et al.*[84] showed that early treatment of OVX rats with alendronate significantly attenuated cartilage erosion by inhibiting subchondral bone loss. Pre-emptive alendronate treatment prevented increased bone turnover, reduced cartilage degradation and preserved the structural integrity of subchondral bone in low-dose monosodium iodoacetate (MIA)-induced knee OA in rats[85].

Treatment with BPs has shown no definite disease-modifying effect in humans; however, several studies revealed a potential benefit of risedronate and alendronate in OA in terms of inhibiting subchondral bone remodelling and improving symptoms and progression of the disease at various locations. Risedronate has been evaluated in phase II and phase III clinical trials, showing positive effects on OA in phase II and negative effects in phase III trials[86–88]. In a 1-year prospective, double-blind, placebo-controlled study of knee OA, Spector *et al.* reported that daily treatment with 15-mg risedronate improved the WOMAC index and patient global assessment (PGA) scores as well as significantly decreased levels of markers of cartilage degradation and bone resorption[86]. Risedronate has also been shown to maintain or increase vertical trabeculae number, thereby preserving the structural integrity of subchondral bone in a 2-year longitudinal radiographic study of knee OA[87]. However, the effects of risedronate on the symptoms of knee OA were inconsistent. In a phase III parallel trial, risedronate failed to show any disease-modifying effect in patients, even though the levels of C-terminal crosslinking telopeptide of type II collagen (CTX-II), a marker of cartilage degradation, were reduced[88,89].

In a cross-sectional study (CS), Carbon *et al.* reported that alendronate decreased the prevalence of knee OA-related subchondral bone lesions and bone marrow oedema[90]. A 2-year follow-up study of alendronate treatment in hip OA showed improved WOMAC pain scores and visual analogue score (VAS) with decreased levels of the NTX-I and CTX-II markers and no improvement in joint space narrowing by X-ray[91]. Use of a sufficient dosage is important to assess the efficacy of BPs in OA, which might be applicable in alternative approaches. Intra-articular clodronate provided symptomatic and functional improvements including, higher VAS and Lequesne index score[92]. A study of the effect of zoledronic acid in osteoarthritic knees suggested that intravenous zoledronic acid (5 mg) reduced knee pain and BML size over a 6-month period[93]. Finally, BPs may be useful in the early stages of OA due to high subchondral bone remodelling observed in clinical trials; the BP doses used must be sufficient to prevent osteochondral osteoclast activity.

Calcitonin

Salmon calcitonin (sCT) is approved for the treatment of osteoporosis and other diseases involving accelerated bone turnover by reducing osteoclastic resorption directly[94]. Oral sCT treatment counteracted the loss of cartilage thickness and reduced subchondral bone damage and type II collagen degradation in a combined meniscectomy with ovariectomy in OA rat models[95]. Behets *et al.* reported the chondroprotective effect of sCT in a canine ACLT OA model. sCT administered intranasally reduced both subchondral bone remodelling and cartilage lesions in the early stages of OA[96]. Other studies reported beneficial effects following prophylactic and therapeutic intramuscular administration of sCT in cartilage lesions and bone turnover in the surgically induced OA rabbit model[97]. In a canine ACLT OA model[98], subcutaneous injections of calcitonin under therapeutic conditions also reduced the progression of osteoarthritic cartilage and subchondral bone changes as well as the levels of serum markers of bone resorption. Recently, Ryan *et al.* reported that an intra-articular sCT-based nanocomplex showed significant anti-inflammatory effects by reducing the mRNA levels of the inflammation marker NR4A2[99].

In clinical trials, Manicourt and Bagger *et al.* reported oral sCT significantly decreased joint pain and function scores as well as levels of bone and cartilage degradation biomarkers[100,101]. Similar results were reported in recent studies. Karsdal and colleagues reported the positive effects of a novel sCT oral formulation delivered with 5-CNAC on bone resorption and cartilage degradation in terms of the reduction in the levels of the CTX-I and CTX-II[102]. Additionally, nasal sCT treatment in knee OA showed positive chondroprotective effects based on improved WOMAC index, VAS and serum NO[103]. A 1-year follow-up clinical trial of nasal sCT treatment showed similar results and exhibited dual effects on osteoporosis and knee OA with significant improvements in quality of life[104]. Additional in-depth phase III clinical trials and subsequent analyses are needed to investigate the potential positive effects of calcitonin on the pathogenesis of OA.

Strontium ranelate

Strontium ranelate (Sra) is an agent with dual effects on bone metabolism in postmenopausal osteoporosis based on the coupling of decreased bone resorption and increased bone formation[105]. A

series of studies indicated this drug might also have beneficial effects in OA. Sra increased human cartilage matrix formation *in vitro*[106]. This drug also stimulated OPG and inhibited RANKL synthesis in human OA subchondral bone osteoblasts, and reduced MMP2 and MMP9 expression[107]. Pelletier *et al.* demonstrated that Sra reduced subchondral bone thickening and articular cartilage lesions by inhibiting key metalloproteases and IL-1β in a canine ACLT OA model[108].

Recently, several studies have explored the clinical efficacy of Sra in OA. In a *post hoc* trial in females with osteoporosis and concomitant spinal OA, oral Sra reduced both the progression of radiographic features and back pain[109]. Alexandersen *et al.* reported that Sra reduced the urinary levels of the cartilage turnover marker CTX-II[110]. Additionally, Reginster *et al.* demonstrated that Sra (2 g/day) provided a beneficial effect on radiographic progression based on joint space narrowing and greater reductions in WOMAC score and the pain subscore in a large, 3-year, randomised clinical trial[111].

Anti-cathepsin K

Cathepsin K is the main osteolytic cysteine protease secreted by osteoclasts, and it plays a critical role in the degradation of the bone matrix and type I collagen[112]. Cathepsin K inhibitors have been suggested to prevent both bone loss and cartilage degeneration. Two novel cathepsin K inhibitors were shown to hinder the progression of OA and reduce joint pain in a canine partial medial MNX OA model and a guinea pig spontaneous OA model[113,114]. Recently, Hayami *et al.* reported that inhibition of cathepsin K prevented ACLT-induced OA in rabbits and mice, showing beneficial effects on subchondral bone, cartilage and osteophytes[115]. This result was in contrast to a study employing the human tumour necrosis factor–transgenic mouse model in cathepsin K-deficient mice, in which a partial protective role in arthritic bone destruction was identified[116].

Bone-forming agents: parathyroid hormone (PTH)

A number of *in vitro* and *in vivo* studies have indicated that PTH, known as teriparatide in drug form, influences articular cartilage homeostasis and may be chondroprotective. PTH[1-34] inhibited expression of COL10 and stimulated expression of COL2 in MSCs from OA patients[117]. Sampson *et al.* reported that teriparatide protected against cartilage degeneration and induced matrix regeneration in a mouse model of injury-induced OA[118]. Chang *et al.* demonstrated that intermittent injection of PTH[1-34] inhibited terminal differentiation of human articular chondrocytes *in vitro* and reduced OA progression in rats[119]. Additionally, they developed PTH/PLGA microspheres, which cause sustained release of PTH, resulting in suppression of OA progression in a papain-induced OA rat model[120]. Chondrodestructive effects upon exposure of articular cartilage to PTH have been reported. Brennan *et al.* showed that higher levels of PTH were detrimental to cartilage and reduced its healing properties following minor injury in humans *in vivo*[121]. Recently, PTH-induced alterations of normal subchondral bone microarchitecture were investigated, and found to result in cartilage damage and provoke early osteoarthritic changes[122]. Bellido *et al.*[123] demonstrated that the improvements in the microstructure and integrity of subchondral bone due to PTH[1-34] may prevent cartilage damage progression in rabbits with early OA preceded by osteoporosis, underlining the intimate crosstalk between articular cartilage and subchondral bone. Intermittent PTH[1-34] administration also ameliorated synovial changes associated with cartilage damage[124]. Although further research is warranted, studies of bone-forming agents can provide novel targets in subchondral bone remodelling for the treatment of OA.

Conclusion and perspectives

The bone–cartilage interface is a biocomposite functional unit that has attracted interest for its uniquely synergistic and cooperative function in OA. Due to the close physical association between cartilage and subchondral bone many physical and functional alterations can occur through molecular interaction across the interface. Elevated molecular transport was identified in osteoarthritic joints based on osteochondral angiogenesis and bone remodelling. The presence of vascular invasion from subchondral bone into the cartilage zone and the existence of microcracks and fissures, and subchondral bone remodelling, provide pathways of communication across the osteochondral interface.

Recent studies *in vitro* and *in vivo* have shown that crosstalk affects cartilage and subchondral bone in synovial joints. Several critical biological factors and signalling pathways, including HIF-2α, OPG/RANK/RANKL, TGF-β and Wntβ-catenin, in both tissues may play regulatory roles in OA progression and the homeostasis of neighbouring tissues. The role of the crosstalk at the bone–cartilage interface in the progression of OA requires additional evidence at the molecular level using genomics, epigenetics, proteomics and metabolomics approaches. An increasing number of potentially effective target candidates for OA therapy, including agonists or inhibitors of molecules and knockout of specific genes, may lead to breakthroughs in OA research and therapy.

Increased understanding of crosstalk between the cartilage and bone in joints during OA will result in new treatments that inhibit or protect the involved pathways. Targeting angiogenesis, neurogenesis and subchondral bone remodelling to decrease the molecular interaction may help to identify novel therapeutic strategies for treatment of OA. Several approaches, such as liposome and nanoparticle systems for local intra-articular delivery of agents, to the treatment of OA are under development. In conclusion, extensive cellular and molecular studies of crosstalk at the bone–cartilage interface will aid our understanding the pathophysiology of OA and improve existing therapeutic strategies. Advances in research and technology are facilitating the development of novel drugs and agents that specifically block mechanisms of crosstalk responsible for the structural changes in osteoarthritic joint tissue, and should be evaluated in clinical trials.

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Competing interests

The authors declare no competing interests.

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