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Targeting TGFβ Signaling in Subchondral Bone and Articular Cartilage Homeostasis

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Abstract

Osteoarthritis (OA) is the most common degenerative joint disease, and there is no disease-modifying therapy for OA currently available. Targeting of articular cartilage alone may not be sufficient to halt this disease progression. Articular cartilage and subchondral bone act as a functional unit. Increasing evidence indicates that transforming growth factor β (TGFβ) plays a crucial role in maintaining homeostasis of both articular cartilage and subchondral bone. Activation of extracellular matrix latent TGFβ at the appropriate time and location is the prerequisite for its function. Aberrant activation of TGFβ in the subchondral bone in response to abnormal mechanical loading environment induces formation of osteroid islets at onset of osteoarthritis. As a result, alteration of subchondral bone structure changes the stress distribution on the articular cartilage and leads to its degeneration. Thus, inhibition of TGFβ activity in the subchondral bone may provide a new avenue of treatment for OA. In this review, we will respectively discuss the role of TGFβ in homeostasis of articular cartilage and subchondral bone as a novel target for OA therapy.

Keywords

Osteoarthritis; TGFβ; Subchondral bone; Articular cartilage

Current understanding of osteoarthritis and treatment

Osteoarthritis (OA) is a noninflammatory degenerative joint disease and the leading cause of physical disability[1]. There are approximately 27 million people that are suffering with this disease in the USA alone[2]. OA represents an enormous societal burden that increases greatly as the population ages. Clinically, OA is described by joint pain and functional impairment including tenderness and limitation of movement[3]; pathologically, OA is characterized by degeneration of cartilage, sclerosis of subchondral bone and marginal osteophytes (Glossary) [4]. Preclinical and clinical studies have primarily focused on articular cartilage for

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decades. Various signaling mechanisms have been suggested to be responsible for the degeneration of articular cartilage, including complement C5, hypoxia-inducible factor-2α, syndecan-4, in addition to the well established ADAMTS5 and matrix metalloproteinase 13 (MMP13) [4–13]. Accordingly, a wide array of agents have been designed and tested in different clinical trials, including glucosamine sulfate, chondroitin sulfate, sodium hyaluronan, doxycycline, and MMP inhibitors[14]. Although various levels of efficacy of these interventions have been reported, none of them successfully ceased OA progression or reverse the pathological changes. To date, OA is still treated by medications and life-style modifications to alleviate pain and reduce functional impairment in clinics[14]. OA management guidelines advocate the use of acetaminophen, NSAIDs, serotonin/norepinephrine reuptake inhibitors and opioids. When pain becomes disabling, surgery may be performed, such as arthroscopy, osteotomy, joint resurfacing, or whole joint replacement[15–16].

The dilemma in OA treatment is that targeting of articular cartilage alone may not be sufficient to halt disease progression. Indeed, increasing evidence indicates that articular cartilage and subchondral bone act in concert as a functional unit[17]. Articular cartilage prevents biomechanical damage caused by severe loading, whereas its homeostasis and integrity relies on the biochemical and biomechanical interplay with subchondral bone[17]. Because of the relatively greater stiffness and strength in comparison with the overlying articular cartilage, the subchondral bone absorbs most of the mechanical force transmitted by diarthrodial joints and provides the mechanical support for overlying articular cartilage[18–19]. Relative to the slower turnover rate of articular cartilage, subchondral bone undergoes more rapid modeling and remodeling in response to the changes of the mechanical environment[20]. The reduced ability of subchondral bone to dissipate the load would be expected to alter the stress distribution on articular cartilage and signaling pathways in chondrocytes in maintaining cartilage homeostasis[21]. It is therefore reasonable to consider osteoarthritis not as simply a disease of cartilage. Transforming growth factor β (TGFβ) is a homeostasis regulator for both subchondral bone and articular cartilage, and increasing evidence indicates altered TGFβ signaling is involved in the pathogenesis of OA development. In this review, we describe the role of TGFβ in maintaining homeostasis of subchondral bone, articular cartilage. Alterations of TGFβ signaling in these tissues impair their integrity as a function unit and initiate osteoarthritic pathology. The potential and associated challenges in the development and application of therapy targeting TGFβ signaling are also discussed.

Temporal-spatial activation of extracellular matrix latent TGFβ

There are more than 40 members in the TGFβ superfamily, which is further classified into four major subfamilies[22–23]. The TGFβ subfamily contains three closely related mammalian isoforms, TGF-β1, -β2 and -β3, that all function through the same receptor signaling systems[24–25]. TGFβs are different from other cytokines and factors in that, upon secretion, they are deposited into the extracelluar matrix (ECM) of different tissues in an inactive, latent form. TGFβ is synthesized as a large precursor molecule which forms a homodimer that interacts with two other polypeptides, latent TGFβ binding protein (LTBP) and latency-associated peptide (LAP), forming a complex named large latent complex (LLC). The LAP

is noncovalently linked to active TGFβ, masking the receptor-binding domains of the TGFβ and rendering it inactive[26–29]. Storage of inactive TGFβs in the matrix enables temporal-spatial regulation of TGFβ activation during tissue homeostasis. Precise activation of latent TGFβ is the prerequisite for it to function in the right locations at a specific time. The TGFβ activation process involves the release of the LLC from the ECM, followed by further proteolysis of LAP to release active TGFβ to its receptors[28]. There are distinct mechanisms employed in activation of TGFβ in different tissues such as proteolytic cleavage and interaction with integrins[30–31]. Proteolytic cleavage of LLC and liberation of active TGFβs can be performed by a variety of MMPs, plasmin, plasminogen activators, thrombin, elastase[32–37]. Independently from proteolytic cleavage, interaction between LAP-β1 and thrombospondin (TSP) 1 and the mannose-6-phosphate receptor also promote latent TGF-β1 activation[31, 38]. In platelets, a furin-like proprotein convertase appears to extracellularly activate latent TGFβ1 independently from any of the above mentioned mechanisms[39]. Integrin αvβ5, αvβ6, αvβ8 and an unidentified β1 integrin and possibly αvβ3 integrin have been reported to participate in activating latent TGFβ1[40–42]. Interestingly, all of these identified integrins that be able to active TGFβ share the αv subunit and recognize the same RGD peptide motif of LAP. These data suggest that αv subunit is the key component for TGFβ activation. Targeting the αv-containing integrin mediated molecular pathway could have clinical utility in the treatment of high TGFβ induced disorders.

During tissue injury or remodeling, TGFβs in the matrix are activated and then signal to recruit stem cells for tissue repair. Adult tissues often harbor resident stem cells or progenitor cells for tissue homeostasis[43]. TGFβs, in consultation with the other signals, appear to regulate stem cells' decision in differentiation or self-renew.[44–47]. Mutations in the extracellular proteins that result in premature activation of TGFβs often lead to skeletal disorders such as Camurati-Engelmann disease (CED), Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS) and Shprintzen- Goldberg syndrome (SGS)[48–52]. Constitutive activation of TGFβ is also associated with tissue fibrosis. Thus, appropriate spatio-temporal TGFβ function is clearly crucial for maintaining healthy skeletal tissue.

Activation latent TGFβ in the matrix maintains bone homeostasis during remodeling

Adult bone is a dynamic tissue in constant remodeling that continuously being formed and resorbed. The remodeling process is necessary to maintain the structural integrity of the skeleton and allows the repair of tissue damage and homeostasis of calcium and phosphorous metabolism[53]. This bone remodeling is accomplished by precise coordination of osteoblasts and osteoclasts[54]. Bone resorption and formation do not occur randomly along the bone surface. Rather, they occur at specific anatomical sites and follow a well-defined sequence of events, the bone remodeling cycle, to maintain bone homeostasis[55]. It has been demonstrated that active TGFβ1 that released during osteoclast bone resorption directs the migration of mesenchymal stem cells (MSCs) to form the new bone at the resportion site[56] (Figure 1). The newly recruited MSCs undergo a cell-lineage specific differentiation that is defined by signals in the microenvironment at the resorptive sites. Both physical properties of the fresh resorption site and soluble factors released from matrix contribute to the

differentiation of MSCs. Once the bone mineral matrix is exposed by osteoclast bone resorption, the stiff microenvironment on the rough bare matrix, which lacks a lining of cell coverage, can facilitate the commitment of MSCs into osteoblasts[57]. Also, osteotropic factors including insulin-like growth factor-1 (IGF-1)s and platelet-derived growth factor (PDGF)s released from exposed bone further stimulates the differentiation of MSCs into osteoblast lineage cells.

Accumulating evidence indicates that high levels of active TGFβ in subchondral bone disrupt joint homeostasis and integrity. TGFβ was found to be aberrantly elevated in OA subchondral bone in both human specimen and various animal models[58]. Abnormal subchondral bone structure and degeneration of articular cartilage were observed in transgenic mice in which active TGFβ1 is constitutively expressed by osteoblastic cells[56, 58]. Genetically, gain-of-function of Smad3 mutations has been linked with the incidence of hip and knee OA and early onset of this disease[59–60]. Aberrant elevation of active TGFβ1in subchondral bone is associated with early signs of OA including bone marrow lesions (BMLs)[58]. High levels of active TGFβ1 induce clustering of MSCs/osteoprogenitor in the subchondral bone marrow and formation of marrow osteoid islets. Indeed, OA progression was attenuated in the mouse anterior cruciate ligament transection (ACLT) model when the TGFβ type II receptor was deleted in MSCs[58].

Dynamic changes in bone microenvironment during bone remodeling

It is known that bone marrow has an organized and structured architecture, and the behavior of MSCs is precisely regulated in this highly dynamic microenvironment. MSCs serve to replenish the differentiated compartment of various cell types for bone formation, angiogenesis, adipogenesis and chondrogenesis[61–62, 43, 63–65]. Extrinsic signals in the surrounding microenvironment that are transmitted to the stem cell niche substantially influence MSC self-renewal or differentiation. TGFβ regulates stem cell quiescence through its direct or indirect effects in modulating the bone marrow microenvironment[66]. In the context of different mophogenetic events, epithelial cells undergo epithelial to mesenchymal transition (EMT) which recapitulated under pathological conditions such as fibrosis and metastasis of carcinomas[6768]. Recently, endothelial to mesenchymal transition (EndoMT) has emerged as another possible source of tissue myofibroblasts. TGFβ signaling has been shown to play an important role in both EMT and EndoMT[67, 69]. In a microenvironment with aberrant elevated active TGFβs, MSCs are recruited in the marrow to form osteoid islets and agiogensis. TGFβ was found to be associated with almost all histological characteristics of BML such as less-well mineralized bone, increased marrow perfusion, and marrow fibrosis[70–72]. Increased angiogenesis in OA subchondral bone provide resources of epithelia and endothelia. Whether EMT is involved in TGFβ-induced MSC clustering and BML formation is worthy of further investigation.

Activation of matrix TGFβ in articular cartilage homeostasis

The indispensable role of TGFβ in maintenance of articular cartilage metabolic homeostasis and structural integrity has been well established[73]. TGFβ stimulates early events in chondrogenesis, including chondrogenic condensation, chondroprogenitor cell proliferation

and differentiation[74–77]. It also inhibits terminal differentiation of chondrocytes, thereby blocking cartilage matrix calcification and vascular to maintain extracellular matrix (ECM) integrity[78]. Interruption of TGFβ signaling in the articular cartilage results in loss of proteoglycans and cartilage degeneration[79]. The effects of TGFβ on articular cartilage can be regulated at different levels: activation of matrix latent TGFβ, expression of different receptors as well as downstream intracellular signaling components. Dysregulation of any factor involved in TGFβ signaling transduction may affect cartilage integrity.

The extracellular matrix of cartilage stores abundant latent TGFβ (~300 ng/ml) that fulfill the needs for sufficient supply of active TGFβ[80]. Exogenous active TGFβ has limited effects on articular cartilage[81–83]. Factors that participated in the activation process of latent TGFβ are often found to be disregulated in OA. The expression of LTBPs is upregulated in both mouse OA model and human OA cartilage[84–86]. OA phenotypes are also observed in LTBP-3 knockout mice similar to impaired TGFβ signaling mouse models[87–88]. Most of the factors and mechanisms have been implicated in the activation process of TGFβ in other tissue and organ also applies to articular cartilage, such as MMPs-, Furin, Transglutaminase, Plasmin, TSP-1 and lysophospholipid[32–33, 89–92]. However, the precise context and mechanistic details in activation of cartilage matrix TGFβ are still unclear.

Articular cartilage is a tissue that resistant to mechanical stress and undergoes atrophy with loading deprivation[93]. Physiological mechanical stimulation promotes chondrocyte ECM protein synthesis critical for maintenance of articular cartilage function and integrity[94]. The effect of shear stress induces an increase of protein synthesis in the superficial zone of articular cartilage, which can can be abolished by treating with TβRI specific inhibitor[95–96]. TGFβ also mediate shear force-stimulated chondrocyte proliferation. These findings indicate the important role of TGFβ signaling in the mechanism of chondrocyte mechanotransduction. Combined with the finding that the latent TGFβ can be activated by shearing forces in synovial fluid[97], it is likely that the TGFβ activation process in articular cartilage is directly or indirectly regulated by mechanical stress. The fact that cells can activate TGFβ in their surrounding ECM through integrin-mediated contractile forces[98–99] implicates a potential mechanism of TGFβ activation by chondrocytes in response to mechanical stress. Thus, integrins may mediate chondrocyte-activation of TGFβ, which is known to stimulate expression of integrins[100–102]. In addition, the integrins appears to play a role in mediating chondrocyte response to TGFβ via regulating its adhering capacity to the type II collagen[100–102]. It would be interesting to investigate whether integrins mediate TGFβ activation via cell-matrix interactions and the potential positive feedback loop between them in articular cartilage.

TGFβ expression is upregulated in the early phase of OA which stimulates chondrocyte proliferation and proteoglycan synthesis in attempting to repair injured cartilage[103–104]. Yet the response of chondrocytes to TGFβ also relies on their differentiation status and TGFβ receptors expression conditions[105]. In general, TGFβ signals via heteromeric complexes of two related transmembrane type I and type II serine/threonine kinase receptors that activate smad-dependent gene transcription. Rather than a TβRII unique to its ligand, the TGFβ type I receptors, also termed activin receptor-like kinases (ALKs), act downstream of type II receptors and determine receptor specificity[106]. SMAD2 and SMAD3 are substrates of

ALK5, whereas ALK1 utilize SMAD1, SMAD5 and SMAD8. During degeneration of articular cartilage, TGFβ signaling pathways are dysregulated with differential expression of TGFβ receptors in the chondrocytes. The mRNA level for TGFβ receptor II was dramatically reduced at early stage OA in a rabbit model[107]. Expression of mutant TGFβ type II receptor (TβRII) promotes terminal chondrocyte differentiation[108]. Increased TβRII degradation and down-regulated TβRI expression lead to decreased sensitivity of articular chondrocytes to TGFβ and accelerate OA development[109–111]. Conditional deletion of Smad3 in chondrocytes induced Runx2 expression and ended up with cartilage degeneration[73, 112].

Moreover, TGFβ was found to signal not only via activin receptor-like kinase 5 (ALK5)-induced Smad2/3 phosphorylation, but also via ALK1-induced Smad1/5/8 phosphorylation[113]. These two main intracellular signaling pathways are often found to act in an opposing or even antagonizing fashion[114]. Both OA and the aging process itself change the pattern of TβRI expression[115]. In a shift to dominant usage of the receptor from ALK5 to ALK1, TGFβ stimulates the catabolic pathway in chondrocytes[111]. Therefore, TGFβ may actas a double edged saw; it is anabolic when signaling through ALK5 in maintenance of articular cartilage homeostasis and catabolic when ALK1 expression is upregulated during progression of OA[105]. A specific ALK1 antagonist could be promising to reduce progression of OA.

In addition, TGFβ co-receptors betaglycan (also termed type III TGFβ receptor), endoglin (CD105) and CD109[116] are emerging as important regulators of TGFβ signaling. Endoglin is a transmenbrane glycoprotein that facilitates TGFβ binding to TβRII with preferential recruitment of ALK1[117]. Betaglycan, a homologue of endoglin, has been shown to direct clathrin-mediated endocytosis of TβRI and TβRII, and enhance TGFβ signaling via Smad and MAP kinase pathways[118–120]. Betaglycan also increases the sensitivity of TβRII to its ligands and equalizes the affinities across TGFβ isoforms, thus maximizing TGFβ signaling[121]. CD109 has been identified as a TGFβ co-receptor and inhibiting Smad2/3 signaling by promoting TGFβ receptor internalization and degradation in a Smad7/Smurf2-dependent manner[122]. Thus, Endoglin, Betaglycan and CD109 may also be considered as potential pharmaceutical targets for OA treatment.

High level of active TGFβ in the subchondral bone at onset of OA

The structure of bone dynamically changes in response to mechanical loading, particularly, when joint stability is decreased in patients during aging or with ligament injury or obesity. Recent studies show that osteocytes regulate the dynamic nature of bone through diverse functions[123]. Osteocytes are now recognized as the principal sensors for mechanical loading and are able to transduce mechanical signals into biological responses[124]. The activities of both osteoblasts and osteoclasts are regulated by the signaling molecules that released by osteoblasts and osteocytes such as osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL) and sclerostin[123]. Alterations in the RANKL/OPG ratio are central in the pathogenesis of bone loss. Denosumab, a monoclonal antibody to RANKL used in the treatment of OA, mimics the function of OPG to induce a sustained inhibition of bone resorption[125] and improve the bone structure [126]. The finding that osteocytes express a

much higher amount of RANKL and have a greater capacity to support osteoclastogenesis than osteoblasts and bone marrow stromal cells provides functional evidence that osteocytes control osteoclastogenesis[127–128]. Therefore, elevated osteoclast activity and turnover rate in OA subchondral bone could be one of the responses of osteocytes to aberrant mechanical loading. Indeed, osteoclastic bone resorption in the subchondral bone was significantly increased as early as 7 days post surgery of ACLT OA mice[58]. In parallel, large quantity of active TGFβ1 released to the marrow during subchondral bone resorption recruits nestin$^+$ MSCs to form marrow osteoid islets and angiogenesis[58]. Notably, osteoclastic bone resorption was uncoupled with TGFβ1-induced recruitment of MSCs in the marrow where they undergo aberrant bone formation. Such responses of subchondral bone alter its microarchitecture and functional integrity with articular cartilage[129]. This notion was substantiated by the development of osteoarthritic-like changes in a transgenic mouse model with osteoblastic expression of active TGFβ1[58].

The subchondral bone volume and subchondral bone plate (SBP) thickness fluctuated substantially in ACLT rodent models[130]. In human osteoarthritis joints, SBP is markedly thicker relative to those of healthy subjects. It is likely that the formation of osteoid islets and abnormal bone formation induced by TGFβ1 changes micro-architecture of subchondral bone[58]. The changes in subchondral bone structure and stiffness may diminish structural support for the overlying cartilage (Figure. 2). For example, expansion of 1–2% subchondral bone significantly changes the distribution of articular cartilage stress in a computerized simulation model for human knee joints. The normal function of articular cartilage relies on the structural integrity and biochemical composition of the extracellular matrix, mainly collagen and proteoglycan. In an mechanical active environment, the balance and organization of these extracellualr matrix macromolecules may be disrupted when biomechanical factors in articular cartilage are altered. Indeed, abnormal mechanical stress induced cartilage structural damage and morphological changes such as clefts, proteoglycan loss and collagen breakdown, have been well documented in previous literatures. Cell death, water content and fibronectin content in the cartilage explants were increased in a load duration and magnitude dependent manner[131]. Vigorous cyclic loading leads to cartilage matrix damage such as collagen fiber broken and proteoglycan depletion possibly due to increased MMP-3[132]. Although intermittent articular loading seems to be necessary for normal cartilage metabolism, abnormal loading patterns likely activate TGFβ irregularly which further induce progressive cartilage degeneration[133]. Therefore, the fluctuation of subchondral bone mechanical property inevitably influences its capacity to dissipate the mechanical stimuli from the joint surface and consequently leads to cartilage degeneration in OA.

Modulation of TGFβ activity in subchondral bone as a potential therapy for OA

Several studies of human OA have pointed to subchondral bone as a site for pharmaceutical intervention. Increased osteoclast activity and bone turnover rate are known pathological characteristics of subchondral bone in OA, particularly at early stage. For this reason, the common anti-resorptive medicine, bisphosphonate, has been tested its efficacy for treating

OA in many clinical trials[134]. Though the outcome in human subjects was not as encouraging as in animal OA models[135–139], specific drugs within the bisphosphonate class did show benefit effects in a few human studies. In the most recent prospective 2-year trial, alendronate treatment successfully improved WOMAC pain score, decrease in biochemical markers in hip osteoarthritis patients[140]. Elderly women being treated with alendronate had a significantly decreased prevalence of knee OA-related subchondral bone lesion and associated with a reduction in knee pain[141]. However, in the recent studies, risedronate failed to improve signs or symptoms of osteoarthritis or alter progression of OA, although a reduction in the level of a marker of cartilage degradation was observed[142–143]. Based on these findings, alendronate seems to be more effective than risedronate for treating OA patients. Yet differences in the study design, such as duration of bisphosphonate use, the dose and route of administration may also affect the results. Moreover, X-ray progression of joint space narrowing may not be sensitive enough to be used in end point judgment. More sensitive and reliable parameters such as BMLs should be considered as end point definition. Future more targeted studies are required to appreciate the value of bisphosphonates in treating osteoarthritis.

Although the approved bisphosphonates differ in structure and activity, they all inhibit osteoclast bone resorption[144–145], a process that allows active TGFβ to be released from bone matrix. Aberrantly activated TGFβ signaling in subchondral bone was found to contribute to OA progression. High levels of active TGFβ were detected in subchondral bone through osteoclast bone resorption at the onset of OA in animal models. Inhibiting bone resorption prevented subsequent activation of TGFβ from matrix. This at least partially suggests a rationale for treating OA with bisphosphonates. Indeed, inhibition of TGFβ signaling in subchondral bone attenuated degeneration of articular cartilage in the ACLT OA rodent models[58]. However, as a critical growth factor, TGFβ plays important role in a wide range of biological processes such as growth inhibition, cell migration, invasion, epithelial–mesenchymal transition (EMT) and immune-regulation. Inhibiting TGFβ activity systemically may therefore affect tissue homeostasis in other organs including articular cartilage. Thus, tissue-oriented therapy that specifically inhibits TGFβ activity in subchondral bone would be a novel approach for treating OA.

High levels of active TGFβ alter the microenvironment of subchondral bone, leading to formation of cluster of osteoprogenitors, osteroid islets and increased angiogenesis. Improving the osteogenic microenvironment may help restore coupling by enhancing the osteogenic potential of MSCs during the reversal phase of bone remodeling, as another potential therapeutic approach. As a hormone that developed during evolution for vertebrates to adapt their terrestrial life, parathyroid hormone (PTH) regulates bone remodeling and improves marrow environment by orchestrating signaling of local factors, including TGF-β, Wnts, bone morphogenetic protein (BMP), and IGF-1[146–148]. During the interactions with TGFβ signaling pathway, PTH induces the recruitment of TβRII as an endocytic activator. TβRII directly phosphorylates the cytoplasmic domain of PTH 1 receptor (PTH1R) and facilitates PTH-induced endocytosis of the PTH1R-TβRII complex in downregulation of TGF-β signaling[146]. PTH also stimulates the commitment of MSCs to the osteoblast lineage by enhancing BMP and Wnt signaling[148]. Moreover, PTH has been shown to spatially relocating small blood vessels closer to sites of new bone formation,

likely secondary to PTH-mediated upregulation of VEGFA and neuropilin 1 and 2[149]. In addition, PTH has been shown to induce cartilage matrix synthesis, suppress chondrocytes hypertrophy and reduce progression of OA in different animal models[150–151]. Thus, PTH's beneficial effects on both articular cartilage and subchondral bone implicate its potential to be developed as a pharmaceutical intervention for OA.

Concluding remarks

Articular cartilage and subchondral bone constantly interact as a functional unit during joint movements. TGFβ plays a critical role in maintenance of both bone and articular cartilage homeostasis. Aberrant activation TGFβ1 in the subchondral bone leads to abnormal bone remodeling and formation of marrow osteroid islets. Importantly, the abnormal subchondral bone structure alters the stress distribution on the articular cartilage and results degeneration of articular cartilage. The concept of the wholism is essential for exploring the therapeutic strategies for OA. Improving mechanical properties of subchondral bone and its physiological function is at least equally important to directly targeting articular cartilage. Therapies that attenuate TGF-β signaling, either directly by neutralizing TGF-β activity or indirectly by PTH-mediated modulation of the bone marrow microenvironment, may serve as potential therapies for these joint disorders. OA is a disease of the whole joint. Therefore, pharmacological interventions that improve interaction between subchondral bone and articular cartilage and their homeostasis could be effective disease modifying treatment for OA.

GLOSSARY

ADAMTS	Abbreviation of "a disintegrin and metalloproteinase with thrombospondin motifs". A family of peptidase function to process of procollagens and von Willebrand factors as well as cleavage of aggrecan, versican, brevican and neurocan
Arthroscopy	A minimally invasive surgical procedure in which an examination and sometimes treatment of damage of the interior of a joint is performed using an arthroscope, a type of endoscope that is inserted into the joint through a small incision
Articular cartilage	Cartilage that covers the articular surfaces of bones
Bone remodeling	A lifelong process where maturebone tissue is removed from the skeleton (a process called bone resorption) and new bone tissue is formed (a process called new bone formation). These processes control the reshaping or replacement of bone following injuries like fractures but also micro-damage, which occurs during normal activity. Remodeling responds also to functional demands of the mechanical loading
Bone marrow lesions	Ill-defined hyperintensities seen on short T1 inversion-recovery images and on fat-suppressed proton density and T2-weighted fast spin echo magnetic resonance images

Bone mineral density (BMD)	A medical term normally referring to the amount of mineral matter per square centimeter of bones. BMD is used in clinical medicine as an indirect indicator of osteoporosis and fracture risk
Chondrocyte	The only cells found in healthy cartilage. They produce and maintain the cartilaginous matrix, which consists mainly of collagen and proteoglycans
Collagen	An insoluble fibrous protein of vertebrates that is the chief constituent of the fibrils of connective tissue and of the organic substance of bones and yields gelatin and glue on prolonged heating with water
Complement	The thermolabile group of proteins in normal blood serum and plasma that in combination with antibodies causes the destruction especially of particulate antigens
Diarthrodial joints	The most common and movable type of joint which is characterized by the presence of a layer of fibrocartilage or hyaline cartilage that lines the opposing bony surfaces, as well as a lubricating synovial fluid within the synovial cavity
Elastase	An enzyme especially of pancreatic juice that digests elastin
Extracelluar matrix	Extracellular part of multicellular structure that typically provides structural and biochemical support to the surrounding cells
Fibronectin	A group of glycoproteins of cell surfaces, blood plasma, and connective tissue that promote cellular adhesion and migration
Glycosidases	An enzyme that catalyzes the hydrolysis of a bond joining a sugar of a glycoside to an alcohol or another sugar unit
Matrix metalloproteinase	A group of zinc-dependent endopeptidases that capable of degrading extracellular matrix proteins and process a number of bioactive molecules
Mesenchymal stem cell	Mutipotent stromal cells that can differentiate into a variety of cell types, including osteoblasts, chondrocytes and adipocytes
Mitogen	A substance that induces mitosis
OPG	osteoprotegerin. a secreted member of the TNF receptor superfamily that negatively regulates osteoclastogenesis. It is a soluble decoy receptor of RANKL that inhibits both cell differentiation and function of osteoclasts by inhibiting the interaction between RANKL and RANK
Osteoblast	Cell with single nuclei that synthesize bone
Osteoclast	The large multinucleate cells closely associated with areas of bone resorption

Osteocyte	Cell that is characteristic of adult bone and is isolated in a lacuna of the bone substance
Osteoid	Unmineralized, organic portion of the bone matrix that forms prior to the maturation of bone tissue
Osteoid islet	Pathological changes in the subchondral bone marrow cavity with under-mineralized osteoid-like structure containing osterix^{+} osteoprogenitors, fibrous tissue and vasculature
Osteophyte	A pathological bony outgrowth; bony projections that form along joint margins
Osteoporosis	A condition that affects especially older women and is characterized by decrease in bone mass with decreased density and enlargement of bone spaces producing porosity and brittleness
Osteotomy	A surgical operation in which a bone is divided or a piece of bone is excised (as to correct a deformity)
Plasmin	A proteolytic enzyme that originally found to be able to dissolves the fibrin of blood clots
Plasminogen	The precursor of plasmin that is found in blood plasma and serum; also name as profibrinolysin
Proteoglycan	A class of glycoproteins of high molecular weight that are found in the extracellular matrix of connective tissue. Proteoglycan are made up mostly of carbohydrate consisting of various polysaccharide side chains linked to a protein, and resemble polysaccharides rather than proteins in their properties
RANKL	Receptor activator of nuclear factor kappa-B ligand. A transmembrane protein belonging to the tumor necrosis factor superfamily that specifically binds receptor activator of nuclear factor -kappa Band osteoprotegerin. It plays an important role in regulating osteoclast differentiation and activation
Stem cell	An unspecialized cell population that gives rise to differentiated cells
Subchondral bone	The layer of bone just below the cartilage which provide support for the cartilage of the articular surface
Subchondral bone Plate	The bone structure that immediately beneath the calcified cartilage. which is a 1–3 mm thick plate of corticalized bone that is physiologically and mechanically similar to cortical bone in other skeletal locations, but somewhat less stiff than diaphyseal cortical bone

Synovial fluid	A transparent viscid lubricating fluid secreted by a membrane of an articulation, bursa, or tendon sheath
Thrombin	A proteolytic enzyme formed from prothrombin that facilitates the clotting of blood by catalyzing conversion of fibrinogen to fibrin and that is used in the form of a powder as a topical hemostatic
Thrombosondin	A secreted protein with antiangiogenic abilities

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Highlights

- Articular cartilage and subchondral bone act as a function unit.
- TGFβ maintains homeostasis of articular cartilage and subchondral bone.
- Spatial and temporal activation of TGFβ is the prerequisite for its function.
- Aberrant activation of TGFβ contributes to onset of osteoarthritis.
- Improvement of microenvironment in subchondral bone has potential for OA treatment.

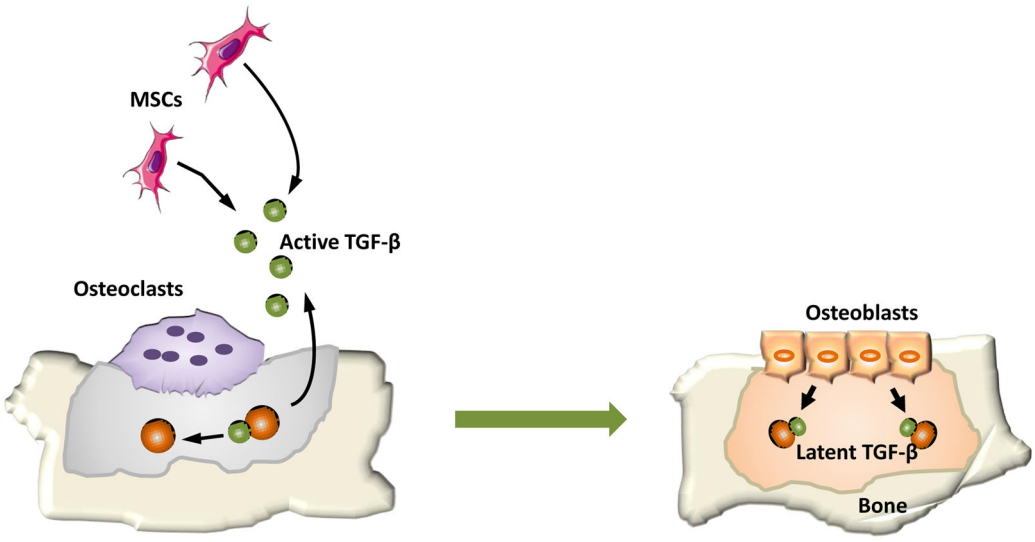

Figure 1. Active TGF-β released during bone resorption coordinates bone formation by inducing migration of bone marrow MSCs (mesenchymal stem cells)

Under normal circumstances, TGFβ is stored in the bone matrix in a latent form. During osteoclast bone resorption, active TGFβ is freed from latent protein and diffuses to the marrow cavity. Following the gradient of active TGFβ, bone marrow MSCs are recruited to the bone resportion site. The MSCs then differentiate to osteoblasts and form new bone to fill the resorbed bone cavities.

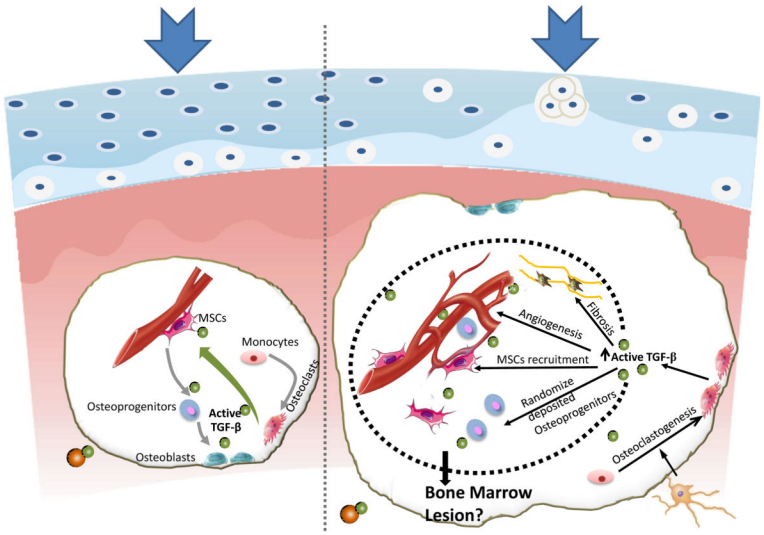

Figure 2. Association of articular cartilage degeneration and pathological changes in subchondral bone at onset of OA

Left panel: Maintenance of homeostasis in articular cartilage and subchondral bone at normal conditions. Right panel: Increased bone turn-over at onset of OA results in elevated active TGFβ levels in subchondral bone. Increased active TGFβ stimulates angiogenesis, marrow fibrosis, clustering of MSC and osteoprogenitors. These cellular pathologies further lead to uncoupled bone remodeling and potentially bone marrow lesion formation. Disrupted architecture of subchondral bone changes its mechanical property and the reduced ability of subchondral bone to dissipate the load contribute to the articular cartilage degeneration.