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SYSTEMATIC REVIEW

The role of TGF-β2 in cartilage development and diseases

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Transforming growth factor-beta2 (TGF-β2) is recognized as a versatile cytokine that plays a vital role in regulation of joint development, homeostasis, and diseases, but its role as a biological mechanism is understood far less than that of its counterpart, TGF-β1. Cartilage as a load-resisting structure in vertebrates however displays a fragile performance when any tissue disturbance occurs, due to its lack of blood vessels, nerves, and lymphatics. Recent reports have indicated that TGF-β2 is involved in the physiological processes of chondrocytes such as proliferation, differentiation, migration, and apoptosis, and the pathological progress of cartilage such as osteoarthritis (OA) and rheumatoid arthritis (RA). TGF-β2 also shows its potent capacity in the repair of cartilage defects by recruiting autologous mesenchymal stem cells and promoting secretion of other growth factor clusters. In addition, some pioneering studies have already considered it as a potential target in the treatment of OA and RA. This article aims to summarize the current progress of TGF-β2 in cartilage development and diseases, which might provide new cues for remodelling of cartilage defect and intervention of cartilage diseases.

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Article focus

- The importance of transforming growth factor-beta2 (TGF-β2) in the physiological activities and pathological states of cartilage has not been fully understood. This article summarizes the current progress of TGF-β2 in cartilage development and diseases, which provides us with a total understanding of TGF-β2, particularly with regards to cartilage defects, degenerative changes, and targeted interventions.

Key messages

- The basal role of TGF-β2 in chondrocyte life cycle is summarized in this study.
- The effect of TGF-β2 on cartilage diseases including osteoarthritis and rheumatoid arthritis is reviewed based on current progress.

Strengths and limitations

- TGF-β2 could regulate chondrocyte behaviours including proliferation, migration, differentiation, and cell death, which makes it a potential therapeutic target in gene therapy, tissue engineering, and stem cell therapy.
- The lack of understanding of the inner biomechanism of TGF-β2 limits its potential applications in both experimental and clinical trials.

Introduction

In recent decades, an increasing number of studies have been conducted on the role of the transforming growth factor-beta (TGF-β) superfamily in physiological and pathological processes of cartilage, especially TGF-β1. However, few studies focus on the role of TGF-β2 in cartilage development and diseases. One study has revealed that TGF-β2 also plays an important role in maintaining homeostasis of cartilage and pathological progress of cartilage.[1] TGF-β2 is known to be a member of the highly conserved TGF-β superfamily, which consists of at least 40 ligand proteins. Moreover, TGF-β2 has two homologous dimeric isoforms: TGF-β1 and TGF-β3, although TGF-β isoforms represent similar structures and different functions.[2] TGF-β2 is a pleiotropic cytokine with important roles in embryonic development

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and tissue homeostasis.[3] In addition, TGF-β2 down-regulated osteogenesis when intermittent pressure was applied to the periodontal ligament cells, meaning TGF-β2 exacted its biological functions depending on ambient conditions.[4] To date, it is known that TGF-β2 participates in the regulation of cell development, wound healing, immune responses, inflammation, and cancer metastasis.[5-9]

Articular cartilage is a highly specialized connective tissue that lives in an avascular, aneural, and hypoxic environment.[10] This connective tissue consists of chondrocytes and extracellular cartilage matrix. Cartilage matrix contains two major organic substances, type II collagen and proteoglycan.[11] Since cartilage tissue lacks blood supply and is a single-cell tissue, the repair ability of articular cartilage is poor.[12] Consequently, it is very important to understand the mechanisms of cartilage defects and explore new potential therapeutic targets in articular cartilage diseases by researching signalling pathways of various cytokines in cartilage. Another study has suggested that TGF-β2 could activate not only canonical drosophila mothers against decapentaplegic protein (Smad) signalling, but also activate noncanonical generic mitogen-activated protein kinases (MAPK) signalling in chondrocytes.[13] For instance, TGF-β2 could induce redifferentiation of chondrocytes by activating activin receptor-like kinase 5 (ALK5)/Smad3 signalling under hypoxic conditions.[14,15] Therefore, TGF-β2 may be a potential therapeutic target for regulating chondrocyte activity and changing the physiological progress of articular cartilage diseases.

Recapitulation

Transforming growth factor-beta2. TGF-β2, a member of the highly conserved TGF-β superfamily, including TGF-betas, activins, and nodal and bone morphogenetic proteins (BMPs), is an indispensable cytokine that can regulate the function of many types of cells, and is involved in the pathological initiation and progression of various diseases.[16-20] TGF-β2, also known as BSC-1 cell (African green monkey kidney epithelial cell) growth inhibitor or polyergin, was first found following the cloning of TGF-β1 in 1985, and another mammalian isoform, TGF-β3, was also found.[21] These TGF-β subtypes exhibit spatial and temporal differences in gene expression, which regulate development of cells and exert profibrotic and anti-inflammatory effects. TGF-β2 is a dimeric protein that consists of two identical disulfide-linked monomers, and its gene has been localized in humans to chromosome 1q41.[22,23] Moreover, the α-helix in TGF-β2 is linked to the remainder of the monomer by an additional disulfide bridge, showing great difference of the structure from the other members of the TGF-β superfamily.[24] Despite these, TGF-β isoforms have many similar peculiarities due to 72% to 80% similarity in the sequence of the active 25 kDa forms. Secreted TGF-β2 is inactive as a high molecular weight latent complex in most cells.[25] Therefore, researching activation mechanisms of latent TGF-β2 is highly important because latent TGF-β also regulates TGF-β biological behaviour.[26] Messenger RNA (mRNA) of TGF-β2 is transferred from the nucleus into the cytoplasm (Figure 1).[27] mRNA of TGF-β2 is translated as pro-protein including TGF-β2 and latency-associated peptide (LAP).[28] Then this pro-protein folds and dimer in the endoplasmic reticulum (ER) with latent TGF-β2-binding protein (LTBP) to form trimeric complex, which is linked to a signal LTBP by a pair of disulfide bonds between LTBP and LAP.[29] The LAP is cleaved in Golgi apparatus by a furin-like convertase, forming the large latent complex (LLC) comprising of TGF-β2, LAP, and LTBP.[30-32] Moreover, the complex of TGF-β2 and LAP is termed as the small latent complex (SLC).[33] After secretion, the LLC can bind to various fibres in the extracellular matrix (ECM) by LTBP.[32] Of course, the LLC of ECM can also be derived from paracrine.[34] Eventually, the LLC is activated by a number of ECM factors, resulting in the formation of active TGF-β2. TGF-β1 and TGF-β3 contain an argine-glycine-aspartic acid (RGD) motif that is recognized by αv integrins, and LLC is activated by integrin αvβ6 in extracellular matrix.[35,36] However, TGF-β2 lacks an RGD motif, resulting in its LLC unable to be activated by integrin αvβ6.[37] This indicates that TGF-β2 may contain other conserved motifs that cannot be found in the other two TGF-βs.[38] Although no studies showed that TGF-β2 could be activated by integrins, many studies have suggested that latent TGF-β2 could be activated by a number of other activators, such as matrix metalloproteinase-2/3 (MMP-2/3) and thrombospondin-1 (TSP1).[37,38] To date, the mechanism by which latent TGF-β2 is activated by various cytokines remains unclear. In the future, further studies of body may determine how to activate latent TGF-β2 and suggest specific activators as drug targets to improve the therapeutic effect of diverse diseases.

TGF-β2 receptors. Activated TGF-β2 delivers signals by binding to its receptors, which are a family of transmembrane protein serine/threonine kinases.[39] TGF-β receptors are divided into three subfamilies: type I (TβRI), type II (TβRII), and type III (TβRIII) receptors, based on their structural and functional properties.[40] These receptors' properties are described as follows:[41-44]

Receptor I: TβRI contains extracellular domain (ECD), which is a specific serine/threonine domain, and the L45 loop, which is a nine amino acid sequence in ECD. Next to ECD, there is a glycine-serine-rich juxtamembrane domain (GS-domain), phosphorylated by the type II receptor kinase. TβRI cannot bind ligands directly but can form a complex with TβRII when TGF-β2 is present.

Receptor II: TβRII has the same domain (ECD) as TβRI. TβRII has sustained kinase activity and can phosphorylate TβRI when these two receptors form complexes, resulting in transmission of the downstream signal of TGF-β2.

Receptor III: β-glycan, which is one of the members of TβRIII, another being endoglin, does not activate protein kinase. β-glycan not only concentrates TGF-β but also stabilizes TGF-β, particularly TGF-β2. However, endoglin selectively binds TGF-β1 and TGF-β3.

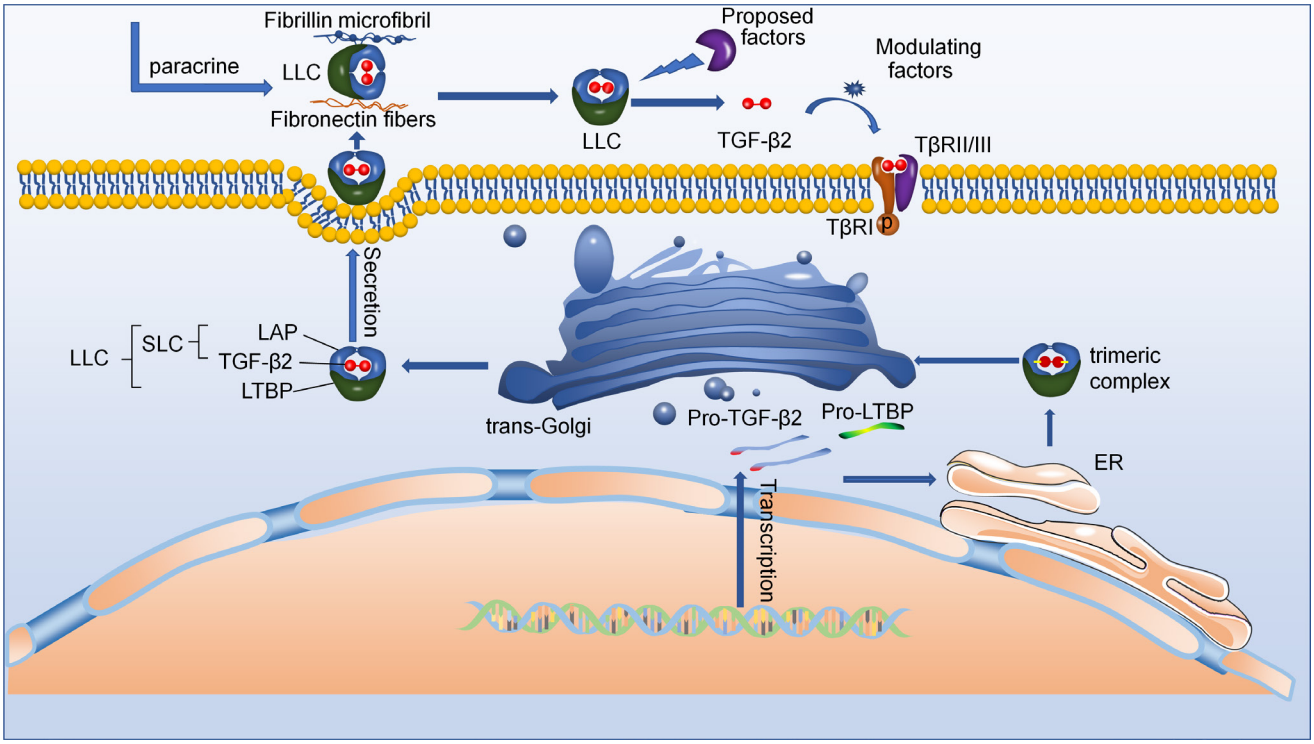

Fig. 1

The schematic diagram showing active transforming growth factor-beta2 (TGF-β2) secretion. Starting at the bottom right: New synthetic pro-TGF-β2 forms dimer/trimeric complexes in the endoplasmic reticulum (ER) with the help of latent TGF-β2-binding protein (LTBP). These dimer/trimeric complexes are then further processed in the trans-Golgi network to form large latent complexes (LLCs). After they are secreted, the LLC may bind to various fibres in the extracellular matrix (ECM) with the help of LTBP. Eventually, the LLC is activated by a number of ECM factors, resulting in the formation of active TGF-β2. LAP, latency-associated peptide.

A great body of evidence suggested that TGF-β2 signals activated canonical ALK5/TβRII/Smad2/3 pathway to exert its functions.[13,44,45] However, in recent research, TGF-β2 has been shown to transduce a signal through noncanonical TβRI/TβRIII/TAK1/p38 pathway to regulate proliferation of palatal mesenchyme, resulting in cleft palate.[46-48] In addition, some research reported that ALK5 could form a complex with other type II receptors to transduce TGF-β2 signal.[49,50] These results indicated that the mechanisms with which TGF-β2 binded to its receptors were highly complex. Therefore, to better understand what affects these mechanisms, further investigation is needed.

TGF-β2 signalling pathway. TGF-β2 can regulate cartilage development and maintenance by its canonical and noncanonical signalling pathways (Figure 2). The LLC is activated by various factors in the ECM, and then active TGF-β2 initiates canonical Smad-dependent signalling pathway.[32] Active TGF-β2 is presented by type III (TβRIII) to its receptors, which are heteromeric complexes of type II (TβRII) and type I (ALK5) receptors.[51] TGF-β2 signals are then transmitted through their downstream signalling pathways that include Smad2 and Smad3.[52] Activated and phosphorylated Smad3/Smad2 form a complex with Smad4 and then translocate into the nucleus.[53] Finally, this complex regulates target gene expression by interacting with other transcription factors in the nucleus.[45] Moreover, active TGF-β2 can also induce the activation of noncanonical MAPK signalling pathways that include p38, extracellular signal-regulated protein kinase (ERK) and Jun N-terminal kinase (JNK), and its receptors are TβRIII/ALK5 complexes that do not contain TβRII.[13] Besides, BMP, a member of the TGF-β superfamily, can activate Smad1/5/8 signalling pathways through TGF-β-activated kinase1 (TAK1) receptor.[54] Studies indicated that BMP signalling pathways played an indispensable role in the early chondrogenesis.[55,56] Interestingly, TGF-β2 signalling pathways generally can resist BMP pathways to maintain cartilage homeostasis by forming mixed Smad3/Smad1/5 complexes, or ski-related novel protein N (SnoN).[57] However, whether and how TAK1 mediates the interaction of TGF-β2 signals and Smad1/5/8 remains unclear.

Smad-dependent TGF-β2 signalling pathway. Smad proteins are canonical intracellular mediators of TGF-β2 signalling pathways, and their name is based on the names of the caenorhabditis elegans (Sma) and drosophila (Mad).[58] Based on the structural and functional characteristics, these proteins are divided into three groups: regulated Smads (Smads1/2/3/5/8), Co-Smad (Smad4), and inhibitory Smads (Smads6/7).[53,59] In addition, there is a unique structure of Smad proteins that contains highly conserved N-terminal (MH1) and CTGF-terminal (MH2) domains and a proline-rich linker region that is of

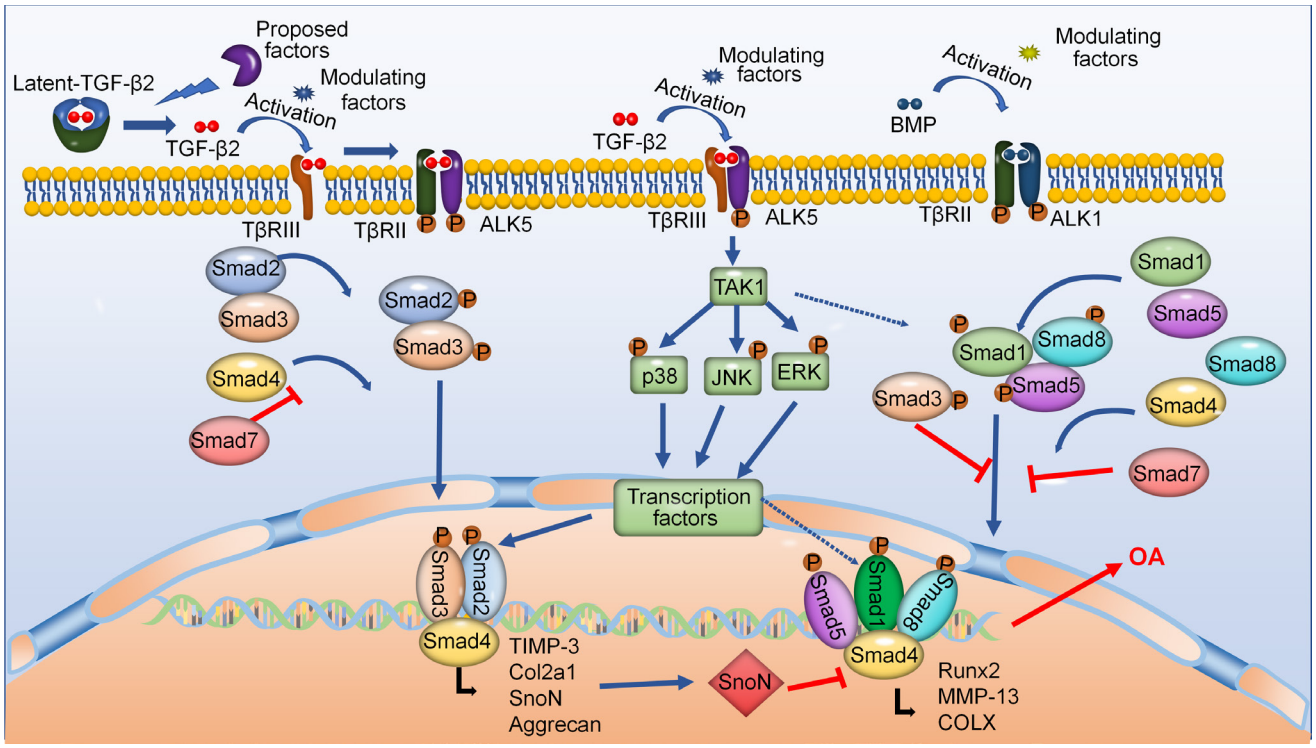

Fig. 2

Transforming growth factor-beta2 (TGF-β2) regulates cartilage homeostasis and disease progression via its canonical Smad-dependent and noncanonical mitogen-activated protein kinases (MAPK) signalling pathways. During the initiation of the canonical Smad-dependent signalling pathway, active TGF-β2 is binded to its receptors, then activates downstream Smad-dependent signalling pathway to regulate homeostasis of chondrocytes. Moreover, TGF-β2 signals can also transmit to noncanonical TGF-β-activated kinase1 (TAK1)-mediated pathways. Additionally, TGF-β2 signals are involved in bone morphogenetic protein (BMP) signalling pathways. ALK1, activin receptor-like kinase; Col2a1, collagen, type II, alpha 1; COLX, collagen type x; MMP-13, matrix metalloproteinase-13; OA, osteoarthritis; TβR, transforming growth beta receptor; TIMP-3, tissue inhibitor of metalloproteinase 3.

divergent length and sequence.[60] These Smad proteins' properties are described as followed:[61-68]

R-Smad: The highly important structure of the MH1 domain that is for binding to DNA are the β-hairpin and nuclear localization signals (NLS). A unique insert in Smad2-MH1 domain, which corresponds to exon. The carboxy-terminal MH2 domain contains a nuclear export signal (NES) that inhibits Smad protein from migrating to nucleus, the L3 loop structure (a 17 amino acid region) that can bind to the type receptors, and the Ser-Ser-X-Ser sequence (SSXS motif) that can be phosphorylated by the type I receptor kinases. The proline-tyrosine-rich (PPXY) motif in the linker domain is recognized by the tryptophan-tryptophan (WW) domain of Smurf family proteins.

Co-Smad: Smad4 encompasses the nuclear export signal at the MH1-linker border, the Smad activation domain (SAD) at the linker-MH2 border, and the L3 loop of MH2 domain.

I-Smad: I-Smads lack the MH1 domain and the SSXS motif, retain a conserved MH2 domain that includes the NES and the L3 loop, and negatively regulate signalling through the proline-tyrosine-rich (PPXY) motif.

Upon TGF-β2 ligand binding, the GS domain of TβRI (ALK5) is phosphorylated by TβRII, resulting in a conformational change in TβRI (ALK5) and increasing the binding affinity of receptor for Smad2 and Smad3, which is determined by the L45 loop of TβRI (ALK5) and the L3 loop of the MH2 domain.[62,69] Phosphorylation of SSXS at the MH2 domain of Smad2/3 by TβRI kinase enables the formation of a tripolymer that is the complex of Smad2/3 associated with Smad4 consequently; the tripolymer migrates into the nucleus to regulate gene expression by the β-hairpin of the MH1 domain combining with DNA and the MH2 domain binding to transcription factors.[62,64,70-72] Of course, this progress can be suppressed by Smad7 of I-Smad.[73] In addition, recent studies suggested that the progression of Smad signalling pathway of TGF-β2 migrating to the nucleus can be disturbed by other signalling pathways, including the Wnt or Hippo pathways.[74-76] According to these studies, signal transduction of TGF-β2/Smad pathway is highly complex.

Noncanonical TGF-β2 signalling pathways. Generic MAPKs are serine/threonine kinases that can be activated by various cytokines, and their signalling pathways are shared by four distinct cascades that include ERK1/2, JNK1/2/3, p38-MAPK, and ERK5.[44,77,78] In recent years, however, studies indicated that the α and β subtypes of p38 were activated by TGF-β2 ligand.[79,80] Furthermore, other studies showed that other signalling pathways of MAPK, such as ERK and JNK, were also activated by TGF-β2/TβRI/TβRIII/TAK1 signalling to regulate development of various cells and

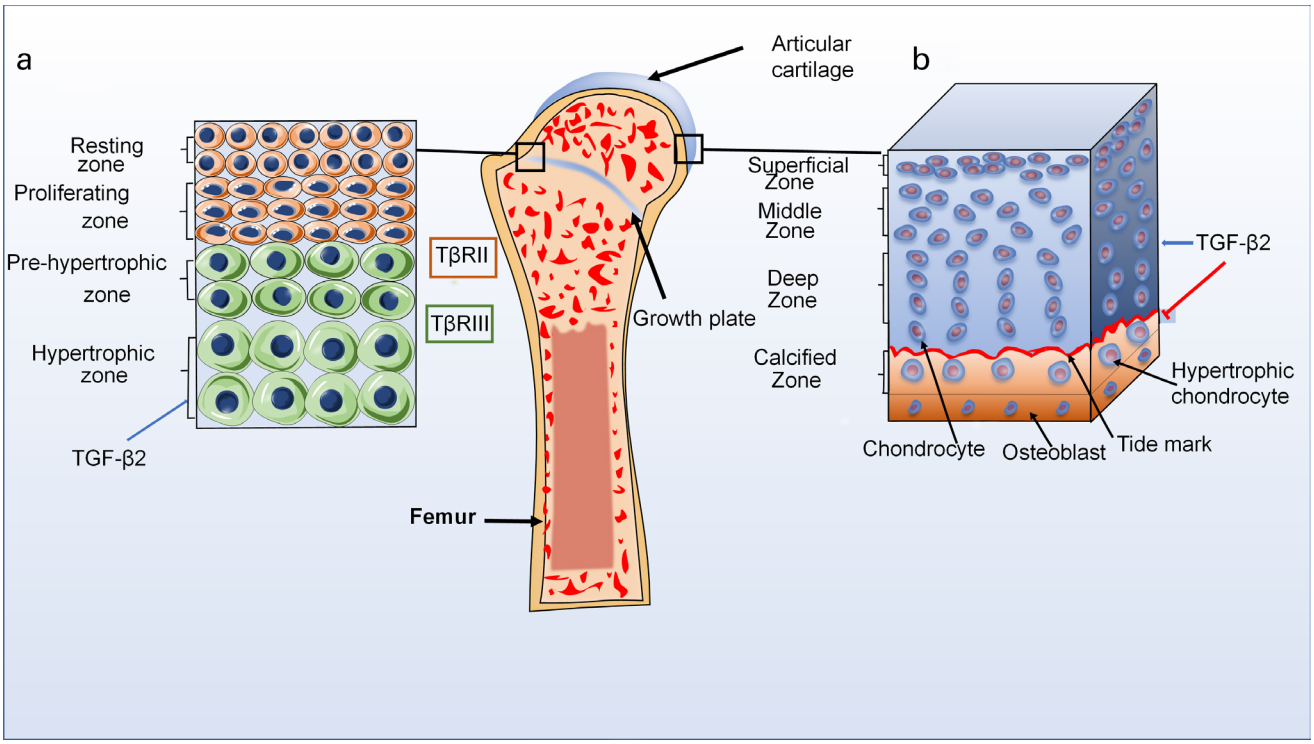

Fig. 3

The distribution of transforming growth factor-beta2 (TGF-β2) in normal articular cartilage. a) Normal structure diagram of growth plate (left). The growth plate cartilage is divided into four zones: resting zone, proliferative zone, pre-hypertrophic zone, and hypertrophic zone. TGF-β2 can be expressed in all zones during cartilage development, but the highest levels are in hypertrophy zone. Interestingly, TGF-β2 has low affinity for transforming growth factor beta receptor (TβR)II but a strong affinity for TβRIII. b) Normal structure diagram of articular cartilage (right). The articular cartilage is also divided into four zones: superficial zone, middle zone, deep zone, and calcified zone. TGF-β2 signalling pathways can maintain chondrocyte phenotype, and inhibit pre-hypertrophic and hypertrophic differentiation in articular cartilage.

pathological progression of diseases.[81] Of note, TAK1 can activate and cooperate with Smad1/5/8 of BMP pathways to regulate cartilage development, but whether and how TAK1 mediates BMP signalling during chondrogenesis remains to be further investigated.[44]

The basal role of TGF-β2 in chondrocyte life cycle

Bone formation in vertebrates is divided into endochondral ossification and intramembranous ossification.[82,83] Articular chondrocytes, which do not undergo terminal differentiation, together with growth plate chondrocytes, which are finally replaced by bone in the embryonic stages of endochondral ossification, are known as two types of chondrocytes.[84,85] Based on structural differences, growth plate cartilage has four zones, which are the resting zone, proliferative zone, pre-hypertrophic zone, and hypertrophic zone, and most of the terminally differentiated chondrocytes undergo apoptosis, leaving ECM that is good for the invasion of osteoblasts and blood vessels (Figure 3).[86,87] Eventually, osteoblasts secrete bone matrix to replace cartilage matrix and realize ossification. TGF-β2 expresses in all zones during cartilage development, but mainly exists in the ECM of hypertrophy zone.[88] Interestingly, TGF-β2 has low affinity for TβRII but a strong affinity for TβRIII.[89] Besides, Horner et al[88] indicated that TβRII achieved maximum expression in the proliferative zone and minimum expression through the hypertrophic zone.

Furthermore, articular cartilage is divided into four zones that incorporate the superficial (tangential) zone, middle (transitional) zone, deep (radial) zone, and calcified zone.[87] Articular cartilage is composed of chondrocytes that occupy only 2% of the total tissue volume of the articular cartilage, and ECM which is composed of fibres (collagen and elastin), proteoglycans, and glycoproteins.[90] Intriguingly, some studies proposed a concept that the pericellular matrix (PCM) of articular cartilage that surrounded each chondrocyte played a dual role both in normal physiological functions of articular cartilage and in the progression of OA.[91,92] Moreover, Jeon et al[93] showed that TGF-β superfamily might suppress the progression in PCM. Of note, TGF-β2 signals can maintain chondrocyte phenotypes, and inhibit pre-hypertrophic differentiation in the articular cartilage.[13] However, the mechanisms of chondrocyte-PCM-ECM interactions and the role of TGF-β2 in PCM are not well understood. Therefore, TGF-β2 is of great importance to better understand how to maintain homeostasis of articular cartilage and find potential therapeutic targets for various diseases by researching the mechanisms of chondrocyte metabolism and ECM turnover.

Chondrocyte proliferation. Proliferation of chondrocytes plays an important role in the pathophysiological process of cartilage, which includes the maintenance of joint homeostasis and the repair of cartilage tissue defects. However, due to its unique physiological properties, cartilage results in a very poor self-repairing capability when cartilage tissue defects occur.[11,12,94] Therefore, exploring the mechanisms that affect chondrocyte proliferation may provide a new method for cartilage repair. Nevertheless, TGF-β2 showed different experimental results in the regulation of chondrocyte proliferation.

Many studies reported that TGF-β2 was expressed in chondrocytes of proliferative and hypertrophic zones of rat growth plates.[44,88] Furthermore, Zerath et al[95] also reported that systemic infusion of TGF-β2 could alleviate the decrease in chondrocyte proliferation induced by unloading model in rats. This result suggested that TGF-β2 might promote proliferation under certain conditions. Interestingly, in recent studies, TGF-β2 had a negative effect on cell proliferation. For instance, Khaghani et al[96] showed that TGF-β2 exerted an inhibitory effect on chondrocyte cell proliferation and might have synergistic effects on joint chondrocytes with other growth factors or proteins, such as basic fibroblast growth factor (bFGF).[97] Moreover, cyclin B2 (the onset of proliferation) and proliferating cell nuclear antigen (PCNA) are regarded as proliferation markers.[98,99] Tchetina et al[98] showed that the downregulation of cyclin B2 expression had a close relationship with TGF-β2. In addition, TGF-β2 can disturb the BMP/Smad1/5/8 signalling pathway that promotes the proliferation of chondrocytes by activating TGF-β2/TAK1/p38 signalling to induce the gene expression of SnoN and Smad7.[44,57,100] These results suggest that TGF-β2 regulates the mechanisms of chondrocyte cell proliferation in a highly complex manner.

Chondrocyte differentiation. Looking at chondrocytes in terms of time, chondrocyte differentiation can be divided into early chondrogenesis, which expresses the type X collagen, and terminal differentiation, which expresses vascular endothelial growth factor A (VEGFA), MMP13, and secreted phosphoprotein1 (Spp1).[101] Moreover, during the process of endochondral bone formation, the differentiation of chondrocytes is an indispensable phase. Chondrocytes in growth plate and cartilage diseases undergo terminal differentiation.[102] However, not all chondrocytes undergo terminal differentiation, such as chondrocytes of articular cartilage. In adult cartilage, most chondrocytes are in a resting state, which has a very poor differentiation capacity. Occasionally, a few chondrocytes are able to differentiate, and can differentiate into activated chondrocytes and hypertrophic chondrocytes. These types of chondrocytes play important roles in the formation of cartilage and the pathological process of cartilage. Interestingly, TGF-β2 signalling pathways can regulate the process of differentiation of chondrocytes. Therefore, exploring the molecular mechanisms of TGF-β2 regulating chondrocyte differentiation is of great significance for cartilage regeneration and treatment of diseases.

Early chondrogenesis. The process of early chondrogenesis in skeletal development of vertebrates is highly complicated because the stage of early chondrogenesis is regulated by multiple transcription factors and signalling pathways, such as Indian hedgehog (Ihh), parathyroid hormone-related peptide (PTHrP), and TGF-β2.[98,103,104] Some chondrocytes participate in the formation of cartilage growth plates by differentiating into hypertrophic cells, and play primary roles in endochondral ossification.[103] Consequently, early chondrogenesis is regarded as the onset of skeletal development and a precondition for cartilage regeneration.

In recent years, to investigate human early chondrogenesis, many researchers simulated the process by inducing stem cells such as bone marrow-derived mesenchymal stem cells (MSCs) and human adipose-derived stem cells (hASCs) to differentiate into chondrocytes.[105] Moreover, most experiments assessed the cell activity and degree of differentiation of chondrocytes by evaluating the quality of typical cartilage differentiation markers such as collagen, type II, alpha 1 (Col2a1), Sox9, and the small intracellular protein S100.[101,106] These studies showed that TGF-β2 could positively regulate the early phase of chondrogenesis of chondrocytes by the TGF-β2/TβRIII/Smad3 signalling pathway.[104,107] For instance, Kim and Im[108] suggested that the combination of TGF-β2 and BMP7 could enhance chondrogenesis from adipose tissue-derived MSCs (ATMSCs). Han et al[109] indicated that β-IGH3, which was a TGF-β2-induced extracellular matrix protein, was highly expressed in early chondrogenesis in ATDC5 (a chondrocyte cell line). Kim et al[110] suggested that staurosporine could induce chondrogenesis in chick embryos through canonical and noncanonical TGF-β2 pathways, which included the Smad2/3 and p38 MAPK signalling pathways. In addition, Hou et al[111] showed that miR-193b negatively regulated the early chondrogenic markers sex-determining region Y box 9 (Sox9) and Col2a1. This indicated that miR-193b played a negative role in early chondrogenesis. Intriguingly, the inhibition of early chondrogenesis, which was activated by miR-193b, bound to the specific seed sequence of the 3'-untranslated regions (UTRs) of TGF-β2 and TβRIII and repressed the expression of TGF-β2 and TβRIII, resulting in the downregulation of early chondrogenesis.

Terminal differentiation. Chondrocytes differentiate into hypertrophic cells, which is the process of terminal differentiation or chondrocyte maturation. However, not all chondrocytes will undergo terminal differentiation. The terminal differentiation of chondrocytes can be seen in the hypertrophic zone of the growth plate and pathological processes of articular cartilage, such as OA.[102] There is no doubt that the terminal differentiated chondrocytes are the most abundant in endochondral ossification of vertebrates. Endochondral ossification, the major mechanism in vertebrates, supports skeletal development.[83,112] Endochondral ossification begins with the condensation of mesenchymal cells, which is a prerequisite for subsequent chondrogenic differentiation.[113] By highly

expressing various adhesion molecules, such as neural cadherin and neural cell adhesion molecules, cartilage is formed in situ, and upregulates the expression of Sox9 to form a body of collagen II and proteoglycans in extracellular matrix. Next, the cells surrounded in the aggregation centre rapidly proliferate and differentiate into different morphological characteristics of chondrocytes, forming four zones in the growth plate, including the resting zone, proliferative zone, pre-hypertrophic zone, and hypertrophic zone.[86,114] Hypertrophic chondrocytes secrete a large amount of Col10a1 and MMP13, which degrades mineralized matrix and facilitates the invasion of blood vessels.[115] Finally, most hypertrophic chondrocytes of terminal differentiation undergo apoptosis, providing space for invasion of osteoblasts, osteoclasts, and blood vessels and resulting in new bone formation.[114] It can be easily observed that the terminal differentiation of chondrocytes plays a pivotal role in endochondral osteogenesis. Intriguingly, many researchers showed that TGF-β2 could be expressed in all layers of the growth plate but its highest levels resided in the hypertrophic layer.[13,88] These studies indicated that TGF-β2 played an important role in the terminal differentiation of chondrocytes. Moreover, TGF-β2 may repress the expression of markers of the terminal differentiation of chondrocytes that contain Col10a1, MMP-13, and Runx2 by activating their signalling pathways.[98,116,117] For example, TGF-β2 can act upstream of PTHrP, inhibiting the terminal differentiation of chondrocytes to repress chondrocyte hypertrophy.[98,118] In addition, Alvarez et al[119] suggested that TGF-β2 could facilitate Sonic Hedgehog (shh) to block terminal differentiation in vitro metatarsal cultures. Wang et al[13] showed that TGF-β2 down-regulated the expression of MMP13 and Runx2 to repress chondrocyte maturation by ALK5/Smad2/3 and p38/MAPK signalling pathways. These studies all showed that TGF-β2 played a highly important role in blocking the terminal differentiation of chondrocytes in vertebrates. However, this function of TGF-β2 still needs to be confirmed in vivo.

Chondrocyte migration. In recent years, many therapies have been used to repair cartilage defects.[108,120] However, surgical interventions often promote the formation of inferior fibrocartilage, which lack the mechanical properties of hyaline cartilage.[121] Additionally, autologous chondrocyte implantation (ACI), a new therapy, has weak long-term effects and leads to defects in donor sites.[122] With the development of cartilage tissue engineering, stem cells from different sources, such as bone marrow mesenchymal stem cells (BMSCs) and adipose tissue-derived MSCs (ATMSCs), are used to regenerate articular cartilage. Although endogenous stem cell regeneration technique of articular cartilage has made great progress, it still faces great challenges, as human-induced pluripotent stem cell (hiPSC) sources are limited and the induction mechanisms of hiPSCs are not well known. Furthermore, migration of hiPSCs to repair sites is a prerequisite step in articular cartilage regeneration. Consequently, it is very important to research how growth factors/chemokines induce stem cells to migrate to cartilage damage sites to repair defects.

At present, the most commonly used cell sources of hiPSCs in cartilage tissue engineering are BMSCs.[123,124] Further, ATMSCs have attracted considerable attention because this type of stem cell is abundant and readily available.[125] Lee et al[126] suggested that MSCs could differentiate into chondrogenic lineage and maintain phenotypic stability during multiple passages. Therefore, the pivotal step is to induce endogenous MSCs to migrate to the site of cartilage defects to facilitate articular cartilage. Studies indicated that TGF-β2 could induce C3H10T1/2 cell (a multipotent mesenchymal precursor cell line) migration at the highest concentration.[127] In all-trans-retinoid acid (ATRA)-treated C3H10T1/2 cells, the chemotactic function of TGF-β2 was significantly improved in comparison to untreated cells. Interestingly, the mRNA expression of TβR2 was obviously decreased in ATRA-treated cells, but levels of TβR1 and TβR3 decreased slightly compared with untreated cells. These results indicated that TGF-β2 might lead C3H10T1/2 cell migration through TGF-β2/TβR1/TβR3/MAPK signalling pathway. In addition, Wang et al[128] suggested that TGF-β2 might induce human marrow-derived mesenchymal progenitor cells to differentiate into chondrocytes. In this experiment, the transfected progenitor cells, which were recombined by transfection of pcDNA3.1(+)/TGF-β2 into BMSCs, upregulated the expression of collagen type II and aggrecan. Moreover, Jin et al[105] showed that TGF-β2 also played an important role in regulating the differentiation of stem cells into chondrocytes. After transfection with a replication-deficient adenovirus carrying h-TGF-β2 (Ad5-h TGF-β2) into adipose derived stem cells (hASCs), the recombinant stem cells were seeded onto poly (DL-lactic-co-glycolic acid) (PLGA)/alginate compounds. The result revealed that the expression of chondrocyte marker genes, Col2a1, and aggrecan was significantly increased in Ad5-h TGF-β2-transduced hASCs and the expression of type I collagen showed a downward trend in comparison to control group. Therefore, these findings indicated that the role of TGF-β2 in promoting cartilage defect repair was very important. On the contrary, Khaghani et al[96] indicated that TGF-β2 repressed the wound healing process of chondrocytes by scratch assay. This meant that TGF-β2 might have a negative regulatory effect on chondrocyte migration. Consequently, more research is needed to better understand the mechanisms of TGF-β2 on chondrocyte migration.

Chondrocyte death. The major forms of death of chondrocytes, which include apoptosis, necrosis, and autophagy, are very common in OA.[129] Of note, TGF-β2 may be involved in the regulation of these three major methods of chondrocyte death. Apoptosis of chondrocytes, which is very important in endochondral ossification, can be induced by TGF-β2/TAK1/p38 MAPK cascade in conjunction with the Smad-dependent pathway.[13] Gibson et al[130] showed that chondrocyte apoptosis was up-regulated by TGF-β2 activation in a dose-dependent manner. In several

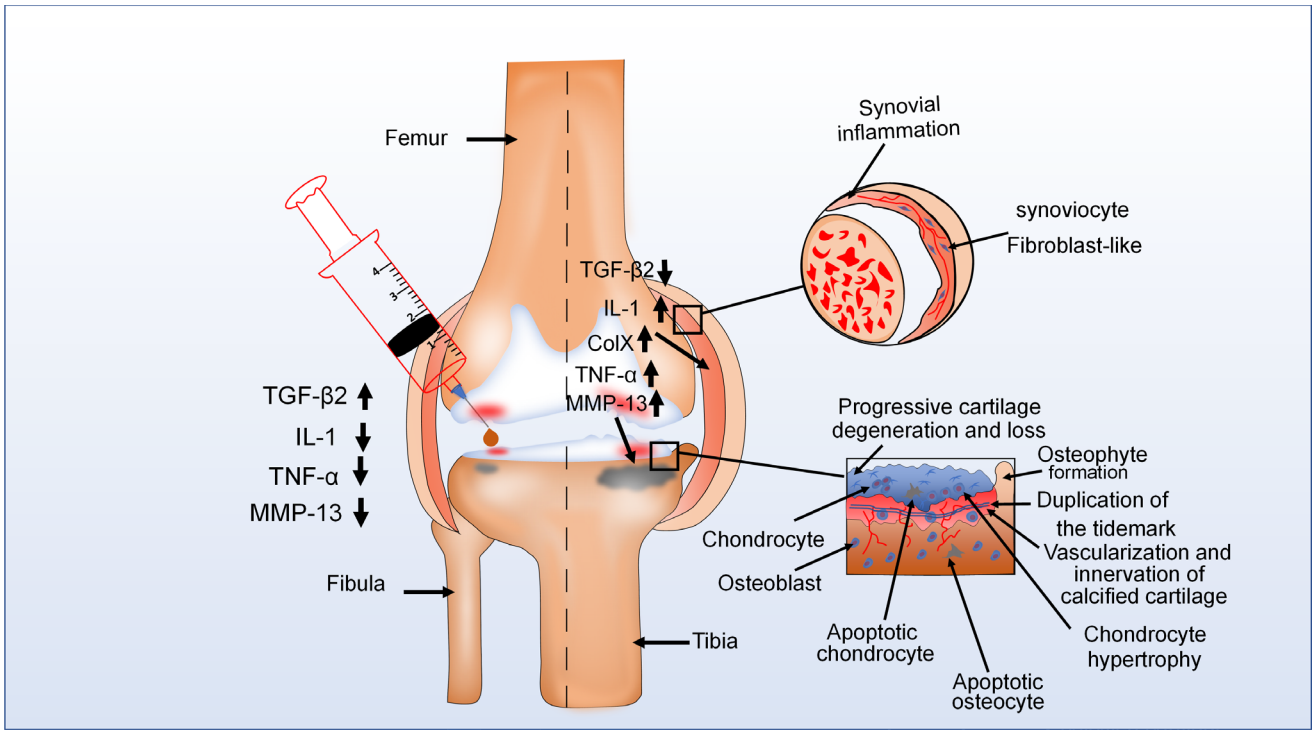

Fig. 4

The pathological change of knee joint in the osteochondral unit during the evolution of osteoarthritis (OA). OA is typically characterized by cartilage damage, osteophyte formation, and thickening of the joint capsule. For example, in one diagram (upper right), the epithelial lining of the joint capsule is thickened because of synovial inflammation. In another (bottom right), during advanced stages of OA, there are pathological changes in different areas of articular cartilage. Moreover, in the left diagram, injection of transforming growth factor-beta 2 (TGF-β2) into the joint cavity of patients with OA reduced the expression of some proinflammatory factors and the clinical symptoms of OA. This means that TGF-β2 plays an important role in the development of OA. ColX, collagen type X; IL-1, interleukin 1; MMP, matrix metalloproteinase; TNF-α, tumour necrosis factor alpha.

inhibitors, SB203580, a specific inhibitor of p38 kinase, completely suppressed TGF-β2-induced caspase.[131] In addition, small interfering RNA (siRNA) knockdown of tumour necrosis factor receptor-associated factor 6 (TRAF6), which was an upstream activator of TAK1, efficiently inhibited apoptosis by TGF-β2/TAK1/p38 MAPK signalling pathway.[132]

In previous studies, death of chondrocytes by apoptosis was associated with severity of OA.[133,134] Moreover, autophagy can protect chondrocyte apoptosis.[135] By studying the regulatory mechanisms of autophagy in chondrocytes, new therapeutic targets to ameliorate the progression of cartilage diseases are found. Recently, many studies reported that TGF-β2 regulated autophagy in different types of cells. For instance, TGF-β2 initiated autophagy to promote glioma cells invasion by Smad and non-Smad pathways, and knockdown of Smad2 or inhibition of c-Jun NH2-terminal kinase decreased TGF-β2-induced autophagy.[136–138] Moreover, Wu et al[137] showed that TGF-β2-treated human retinal pigment epithelium (RPE) cells (ARPE-19 cell line) exhibited increased autophagic flux compared with control cells. Interestingly, TGF-β2-induced EMT (epithelial to mesenchymal transition) is dampened by inhibiting autophagy.[138] These results showed that the role of TGF-β2 signalling pathways in regulating the autophagy of various cells might be consistent and that the process was complex. However, the role of TGF-β2 in regulation of autophagy in chondrocytes has not been studied well yet. Studying the regulation mechanism of TGF-β2 in chondrocytes autophagy, therefore, may provide new therapeutic targets to ameliorate the progression of cartilage diseases.

Effect of TGF-β2 on cartilage diseases

The ECM, synthesized by chondrocytes, together with chondrocytes make up the structure of cartilage. The activity of chondrocytes plays a significant role in maintaining homeostasis of cartilage tissues. Furthermore, aetiological factors, such as mechanical trauma, degeneration of ageing, genetic predisposition, can destroy the homeostasis of cartilage tissue. However, the self-repairing capability of cartilage tissue is very poor, resulting in failure to regenerate after destruction.[139] If effective treatments are not taken, more serious consequences will result. Therefore, many researchers are searching therapeutic methods by exploring the pathological process of cartilage diseases. According to a large number of studies, TGF-β2 plays an important role in the development of cartilage diseases and may serve as a new therapeutic target.[20,140-142] The following sections describe the frontiers of common cartilage diseases and their relationship with TGF-β2.

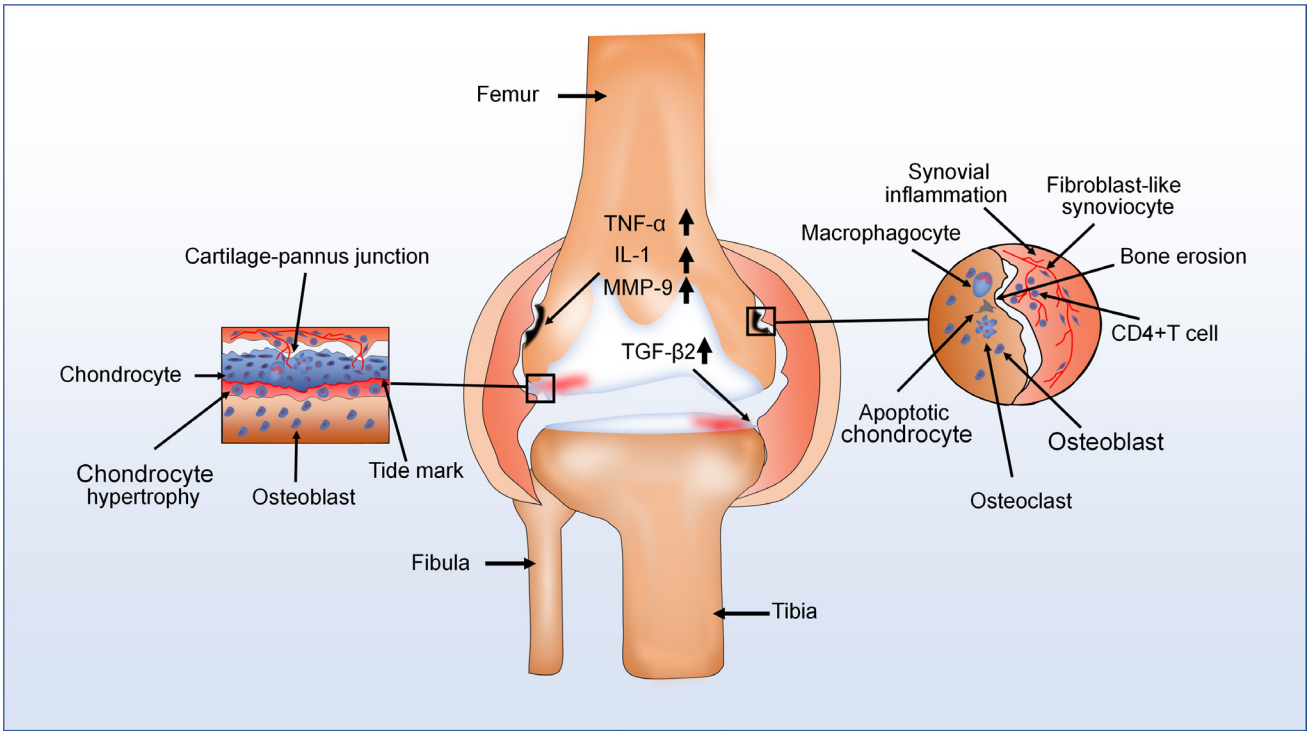

Fig. 5

The major pathological characteristics of rheumatoid arthritis (RA) are chronic synovitis with hyperplasia, pannus formation, and immune cell infiltration. For instance, in one diagram (left), various cell types in the cartilage-pannus of RA secrete lots of proinflammatory factors to induce cartilage damage and degradation of collagen. In another (right), there are also numerous immune cells infiltration and development of new blood vessels in the synovium of RA with hyperplasia, and the expression of transforming growth factor-beta2 (TGF-β2) also increases. Moreover, bone erosion appeared in the vicinity of the thickened synovium. CD4, cluster of differentiation 4; IL-1, interleukin 1; MMP, matrix metalloproteinase; TNF-α, tumour necrosis factor alpha.

Osteoarthritis. OA is the most common and age-related joint disease.[84] Worldwide, more than 15% (10% of men and 18% of women) of the elderly has been diagnosed with OA.[123,143] The common clinical symptoms of OA include long-term chronic pain, joint dysfunction, and deformities.[144] To date, the precise molecular mechanism of the pathogenesis of OA remains unclear. Unfortunately, there are currently no effective therapies to prevent the progression of OA. There are only symptomatic treatments, such as those that relieve pain, ameliorate stiffness, decrease patient suffering, and improve their quality of life. For advanced OA, arthroplasty surgery is the only option, and this therapeutic method hardly achieves satisfactory results. Consequently, in recent years, most researchers have explored the molecular mechanisms related to pathogenesis of OA to identify new potential molecular targets for prevention and treatment.

The major pathological features of OA are progressive hyaline cartilage loss, concomitant sclerotic changes in the subchondral bone, synovitis, and the development of osteophytes.[20,145] During the advanced stage of OA, the epithelial lining of the joint capsule is thickened due to synovial inflammation, causing most new blood vessel formation and fibroblast-like synoviocyte proliferation (Figure 4). Meanwhile, there are high levels of interleukin-1 (IL-1) in synovial fluids of OA, and the expression level of TGF-β2 significantly decreases.[146] In addition, pathological changes occur in different areas of articular cartilage. For instance, various proinflammatory factors such as IL-1, MMP-13, and TNF-α are highly expressed in articular cartilage of OA.[147-149] In the deeper zone, chondrocytes undergo terminal differentiation, which can synthesize collagen type X (ColX), and this accelerates the progression of OA.[94] The calcified zone exhibited several pathological changes, manifested by vascularization, innervation from subchondral bone, and duplication of tidemark.[150] In the subchondral bone, osteocyte apoptosis occurs and osteophytes develop at the joint margins.[151] According to most research, one of the earliest changes in OA was swelling of the cartilage ECM.[152,153] This indicated that disruption of homeostasis in cartilage tissue was one of the major reasons for the initiation of OA. Moreover, the catabolic and anabolic imbalance of chondrocytes, which is due to the change in chondrocyte phenotype, results in the disorder of cartilage homeostasis.[154-156] Thus, the alteration in chondrocyte phenotype may play an important role in the progression of OA. The most common phenotypic change in chondrocytes is hypertrophic phenotype in OA.[157] Interestingly, studies indicated that TGF-β2 could effectively inhibit the progression of OA.[147-149] TGF-β2 not only inhibits the cleavage of Col2 but also suppresses the expression of hypertrophic markers such as Runx2, Col10a1, and MMP-13.[20,158] SnoN, which is induced by the TGF-β2/TAK1/p38 signalling

pathway, can inhibit the expression of MMP13 by BMP/Smad1/5/8 signalling pathway in OA.[13,57] In addition, research also reported that TGF-β2 could promote the expression of specific tissue inhibitors of MMPs (TIMPs) in different types of cells, especially TIMP-3.[140,141] TIMP-3 can protect cartilage tissue by inhibiting MMP-13 degradation of cartilage matrix in OA. TGF-β2 can downregulate IL-1β and TNF-α to inhibit collagenase activity and proteoglycan degradation in OA.[155,159,160] Moreover, TGF-β2 also plays an important role in controlling collagen degradation of articular cartilage by downregulating MMP-9 in OA.[20] Despite the protective role of TGF-β2 in chondrocyte homeostasis in OA progress, studies have shown that TGF-β2 could destroy normal cartilage structure at high concentration. For instance, Elford et al[161] showed that injecting high levels of TGF-β2 into rabbit normal joints caused swelling and significant loss of proteoglycan. This indicated that the regulatory role of TGF-β2 might be dose-dependent in OA. From this evidence, we learn that TGF-β2 can block the pathological changes of OA by regulating the expression of various cytokines, which suggests that TGF-β2 may be a new target for the treatment of OA.

Rheumatoid arthritis. Human rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease associated with pain, joint dysfunction, and other clinical symptoms.[162] The major pathological characteristics of RA are chronic synovitis with hyperplasia, bone erosion, pannus formation, and immune cell infiltration (Figure 5).[163,164] During the progression of RA, a variety type of cells exists in the cartilage-pannus of RA, including monocytes, cluster of differentiation 4 (CD4)+ T cells, and fibroblast-like cells. These cells secrete high level of proinflammatory factors such as IL-1, TNF-α, and MMP9 to induce cartilage damage and degradation of collagen.[165-167] Interestingly, high expression of TGF-β2 is detected in the cartilage-pannus junction during RA.[167] However, little research has been done on the role of TGF-β2 in RA. There is no doubt that TGF-β2 serves an important role in the pathological changes of RA. Müssener et al[168] showed that the expression of TGF-β2 was upregulated with time after onset of disease in the synovial tissue. Müssener et al[168] also indicated that TGF-β2 not only promoted cartilage tissue repair by promoting collagen and fibronectin synthesis, as well as downregulating synthesis of proteases, but also inhibited the entry of lymphoid cells into the arthritic joints in RA. In addition, TNF-α, a major proinflammatory factor in RA, can induce fibroblast-like synoviocytes (FLSs) to produce IL-6, IL-8, and MMPs to cleave collagen of ECM and break down bone tissue.[167,169] According to some studies, evidence suggests that TGF-β2 had an inhibitory effect on TNF-α and IL-1β expression in inflammatory joint diseases.[155,159,160] These results indicated that TGF-β2 might slow down the progression of RA by suppressing TNF-α expression. Han et al[166] showed that gremlin1 (GREM1) was a pivotal regulator of synoviocyte hyperplasia and invasiveness in RA. In this experiment, TGF-β upregulated GREM1 expression in RA-FLS, and could result in synovial hyperplasia. However, this experiment did not prove the effect of various isoforms of TGF-β on GREM1 in RA-FLS. Therefore, whether and how TGF-β2 plays a promotive or inhibitory role in the progression of RA requires further investigation.

In summary, TGF-β2 is involved in the regulation of the entire process of endochondral ossification, such as cartilage, growth plate development, and joint formation. In addition, TGF-β2 also participates in maintaining homeostasis of cartilage tissue and regulates chondrocyte proliferation, differentiation, apoptosis, and the expression of proinflammatory factors. Altering the TGF-β2 levels or using inhibitors can affect the physiological processes of chondrocytes. Consequently, TGF-β2 exerts a significant influence on physiological and pathological processes of cartilage tissue.

Although considerable progress has been made in the study of the role of TGF-β2 in cartilage, many problems have yet to be solved. For example, the effects of TGF-β2 on the initiation and progression of RA remain unknown. So far, most of the research has been carried out on TGF-β1 and TGF-β3, while research into the role of TGF-β2 in cartilage has been very limited. Therefore, the mechanism by which TGF-β2 regulates chondrogenesis and cartilage maturation is unclear. The regulatory mechanisms in the physiological and pathological processes of cartilage mediated by TGF-β2 are also not well understood. In addition, little evidence is provided to explain how TGF-β2 recruits stem cells to repair cartilage defects through signalling pathways. Hence, in the future, more research needs to be done to detect potential mechanisms of TGF-β2 in joint tissues, which will deepen understanding and recognition of the molecular mechanisms of chondrogenesis and be beneficial to the prevention, diagnosis, and therapy of diseases of cartilage tissue.

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