Life Sciences 312 (2023) 121190

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Perlecan: Roles in osteoarthritis and potential treating target

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ARTICLE INFO

Keywords:
Osteoarthritis
Perlecan
Pericellular matrix
Pathogenesis

ABSTRACT

Osteoarthritis (OA) is the most common joint disease, affecting hundreds of millions of people globally, which leads to a high cost of treatment and further medical care and an apparent decrease in patient prognosis. The recent view of OA pathogenesis is that increased vascularity, bone remodeling, and disordered turnover are influenced by multivariate risk factors, such as age, obesity, and overloading. The view also reveals the gap between the development of these processes and early stage risk factors. This review presents the latest research on OA-related signaling pathways and analyzes the potential roles of perlecan, a typical component of the well-known protective structure against osteoarthritic pericellular matrix (PCM). Based on the experimental results observed in end-stage OA models, we summarized and analyzed the role of perlecan in the development of OA. In normal cartilage, it plays a protective role by maintaining the integrin of PCM and sequesters growth factors. Second, perlecan in cartilage is required to not only activate vascular epithelium growth factor receptor (VEGFR) signaling of endothelial cells for vascular invasion and catabolic autophagy, but also for different signaling pathways for the catabolic and anabolic actions of chondrocytes. Finally, perlecan may participate in pain sensitization pathways.

1. Introduction

Osteoarthritis (OA) is characterized primarily by pain and loss of normal articular cartilage function, while synovitis, endochondral ossification, and osteophyte formation are considered secondary phenomena [1,2]. OA initiation and progression are different processes. The early stage is characterized by increased vascularity and reduced bone density, while the late stage is characterized by decreased bone resorption without decreased bone formation [3]. The current view of OA pathology is bone remodeling under risk factors [2], including both systemic and local biomechanical factors such as abnormal bone morphology, sports activity, injuries, and obesity. Some factors can only increase the risk of osteoarthritis, while others can accelerate progression [1]. The diversity of these roles indicates that these factors may act on specific functional substances.

In the extracellular matrix, chondrocytes are surrounded by a pericellular matrix (PCM), which buffers the mechanical load on the chondrocytes and plays a protective role. Meanwhile, all molecules interacting with the surface of chondrocytes must pass through the surrounding environment; therefore, the signal perceived by chondrocytes will be affected by PCM [4–6]. In addition to mechanotransduction functions, it also plays an important mechanobiological role. It has been found to provide a coordinated extracellular environment in normal cartilage structure [7]. PCM remodeling is the initial or progressive factor of OA [8]. PCM and chondrocytes constitute chondrons, whose changes may play an important role in the development of OA [9].

Perlecan, a typical proteoglycan encoded by the HSPG2 gene, is expressed throughout the whole life cycle; it overwhelms the majority of natural tissues and cells and combines with several types of collagens to form a stable structure of PCM, including in the cerebral vasculature [10], skin, liver [11] and cartilage [12]. Perlecan is composed of five

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https://doi.org/10.1016/j.lfs.2022.121190
Received 10 September 2022; Received in revised form 5 November 2022; Accepted 9 November 2022
Available online 12 November 2022

different functional domains and is modified by a variety of sugar chains, which are key modifiers of growth factor transmission [13]. Previous studies have found a crucial scaffold role in development, where partial or complete deletion of HSPG2 can cause death and cartilage dysplasia in mice during pregnancy [14]. Approximately 40 % of perlecan-null (Pln-null) (Hspg2-/–Tg) mice die at embryonic day 10.5 (E10.5) because of poor cephalic development, and approximately 60 % of Pln-null animals die shortly after birth [15]. Other mutants are also associated with Schwartz-Jampel syndrome and dyssegmental dysplasia [16]. Perlecan expression persists in human samples of postnatal, juvenile, adolescent, and adult cartilage, and controls endochondral ossification during the development of cartilage and bone [17]. However, systematic studies of its role in OA are lacking.

Along with the development of OA, there are also apparent increases in the levels of pro-inflammatory factors, which further increase the levels of various proteases, such as MMP13 [18] and glycosaminoglycan proteases (GAGases) [16], corresponding to the perlecan domain IV (Pln IV) and GAG chains, leading to perlecan fragmentation and the release of growth factors. The cleavage of perlecan potentially leads to turnover and functional dysregulation.

This review systematically discusses the changes in perlecan in the pathogenesis of OA combined with the known roles of perlecan in maintaining the structural stability of the external matrix, information transmission, regulation of chondrocyte formation, differentiation, maturation and matrix synthesis, and even fibrosis, to try to analyze its function as a channel linking early risk factors and end-stage osteoarthritis and forecast the treatment prospects based on the pathways.

2. Perlecan distribution and domain-specific interactions

Perlecan is expressed in the BM of a large number of cells and tissues. Its distribution in the cartilage is important to clarify its function in cartilage degeneration and regeneration. Immunolocalization of cartilaginous fetal and mature human tissues demonstrated that perlecan has a strong pericellular distribution around hypertrophic growth plate chondrocytes and throughout the cartilaginous rudiment of the fetal human elbow and knee, except at their margins, and is also present in small blood vessels in the synovial lining tissues, perichondrium, and growth plate. In postnatal juvenile, adolescent, and adult cartilage, perlecan was abundant in the pericellular surroundings of chondrocytes, but it was absent in the more distant intercellular matrix [12].

Perlecan contains a protein core composed of five well-defined domains that interact with various structural or functional components and exert their effects [19] (Fig. 1). Human cartilage extracts from 3- and 65-year-old individuals included many perlecan core protein species, ranging in size from 70 to 381 kDa. Studies have shown that endothelial, epithelial, smooth muscle, bone marrow, and keratinocytes all contain a variety of perlecan core protein species that are comparable in size to those found in the cartilage. Each domain has special structure and affinity for specific factors as listed in Table 1.

Table 1
The protein domains of Pln and their functions through interactive ligands.

Domain	Interacting partner (s)	Potential functions	References
I	VEGF, PRELP, FGF, BMP-2, PDGF, HGF, SHH, WARP, Ang-3, LN, COL IV, V, VI, Xl, XII, XIII, XIV; IL-8, IL-2, NID-1, FN, FBN1, activin A, TSP1	Angiogenesis; Cell adhesion and motility; Chondrogenesis; GF delivery and activation; ECM anchoring, assembly and stabilization	[13,19–28]
II	LDL, CTGF, Hcs24, FBN1, Wnt/calcium	modulate calcium binding and mediate Wnt/calcium signaling; BM anchoring; biogenesis of microfibrils	[13,19,29,30]
III	FGF7, FGF18, WARP	GF delivery; Cartilage structure assembly; ECM assembly/ stabilization	[19,31]
IV	Heparin/sulfatide binding, FGF18, FN, NID1/2, PDGF, COL IV, FBLN-2	cartilage development; BM stabilization	[13,19,24,32–34]
V	NID1, FGF-7, FBLN2, α-DG, PGRN, β1-integrin, COL VI, α2β1-integrin, TGF, VEGF, ECM-1, AchE	Angiogenesis; autophagy regulation; BM assembly/ stabilization; cell proliferation	[13,19,20,28,35,36]

Abbreviation: AchE: acetylcholinesterase; Ang-3: angiopoietin-3; BM: basement membrane; BMP-2: bone morphogenetic protein-2; DG: dystroglycan; ECM: extracellular matrix; ECM1: extracellular matrix protein 1; FBLN2: fibulin-2; FBN1: fibrillin-1; FGF: fibroblast growth factor; FN: fibronectin; GF: growth factor; HGF: hepatocyte growth factor; LDL: low-density lipoprotein; LN: laminin; NID: nidogen; PDGF: platelet-derived growth factor; PGRN: progranulin; PRELP: proline/arginine-rich end leucine-rich repeat protein; TSP: thrombospondin; VEGF: vascular endothelial growth factor; WARP: von Willebrand factor A domain related protein.

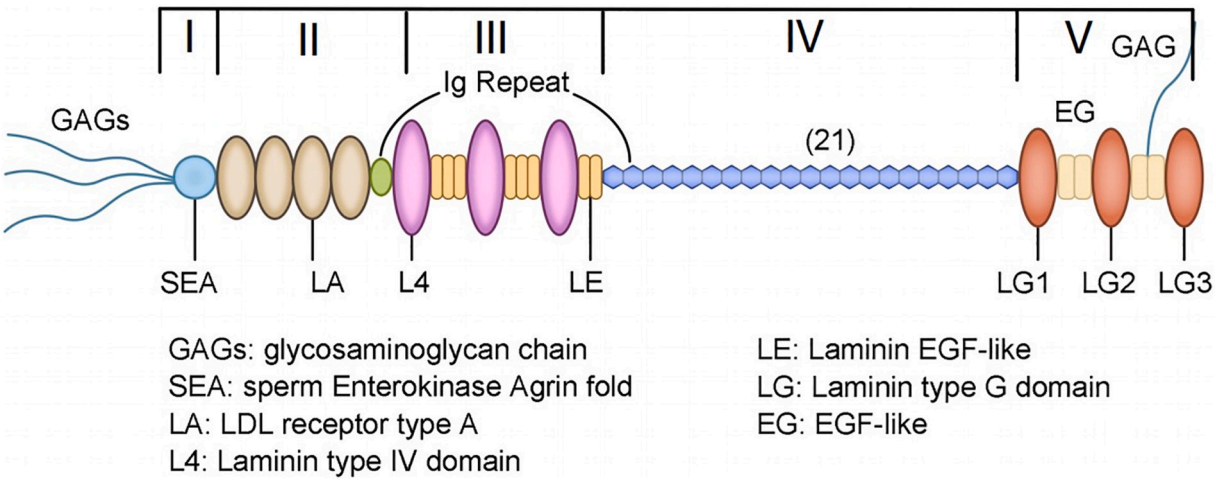

Fig. 1. Schematic illustration of the multidomain structure of human perlecan.

2.1. Perlecan domain I (PlnDI)

PlnDI is composed of a sea urchin sperm protein, enterokinase, agrin module [20] and three potential binding sites where the amino acid sequence can be modified with either heparan sulfate (HS) or chondroitin sulfate (CS) chains, depending on the cell or tissue source and physiological conditions [19,21,22]. The glycosaminoglycan (GAG) chain is crucial for connecting its components and functions.

Through its HS subside chain known for its affinity with some signaling molecules, PlnDI can combine with VEGF, FGF2 [23], FGF18 [24], PDGF, BMP-2, hepatic growth factor, granulocyte-macrophage colony-stimulating factor, and angiopoietin-3 [25], indicating its role in transmitting functional signals to the adjacent cells and tissues. Furthermore, PlnDI can interact with sonic hedgehog (SHH), von Willebrand factor A domain-related protein (WARP), laminin [26], several types of collagen [20,27], nidogen-1 [28], fibronectin, thrombospondin-1, fibrillin-1, IL-2, IL-8, and activin A to stabilize ECM and BM [13,20].

2.2. Perlecan domain II (PInDII)

PlnDII contains four copies of internal repeats that are highly homologous to the low-density lipoprotein (LDL) region of the LDL receptor [19]. It also contains a site that interacts with CTGF/Hcs24 [29] to regulate the proliferation and differentiation of chondrocytes and fibrillin-1 [30] in connection with BM.

Between PlnII and PlnIII, there is a piece of repeat homologous to that of immunoglobulin (Ig) that is also found in PlnIV [19], which can modulate Wnt/calcium signaling [13].

2.3. Perlecan domain III (PlnDIII)

PlnDIII contains four internal cysteine-rich repeat subdomains separated by three laminin-like globular domains [19]. Cationic filtration and immunoprecipitation assays revealed that the binding of FGF-18 to perlecan was not affected by CS or HS digestion but was substantially reduced by the reduction and alkylation of the perlecan core protein. This indicated that the perlecan core protein (and not the CS or HS chains) is involved in FGF-18 binding. Further investigations showed that low-affinity binding sites for FGF-18 are present in PlnDIII [31].

2.4. PlnIV

PlnIV is composed of 21 consecutive Ig-like repeats, similar to those found in neural cell adhesion molecules. HS binding is restricted to IG5 [32,33], which is a potential site for FGF18 [24]. Fibronectin binding is localized to IG4-5 [32,33]. The 14th Ig-like repeat contains a potential GAG chain attachment sequence [13,19]. Fibulin-2 binds to IG2 and IG13-15 with different affinities [32,33]. It is of great importance in cartilage development as it triggers chondrocyte condensation and BM stabilization through interactions with fibronectin, nidogen-1/2, COL IV, fibulin-2, and PDGF [34].

2.5. Perlecan domain V(PlnDV)

PlnDV contains three laminin-like globular domains, LG1, LG2, and LG3, which are separated by two EGF-like domains. The three globular domains are similar to the A chains of laminin [13,19], and are weaker candidates for GAG attachment than domain1 [19]. If it is modified by HS, it will be blessed with a similar, but, to some extent, lower affinity like PlnI with PRELP, nidogen-1, FGF-7 [35], fibulin-2 [28], COLVI, and TGF-beta. It can also interact with ECM protein 1 [36] involved in bone formation and angiogenesis, α-dystroglycan, progranulin, β1-integrin promoting cell adhesion [20,28] and acetylcholinesterase.

Table 1.

3. Biomechanical functions of perlecan and related structure changes in OA

PCM is composed of components similar to the ECM, such as proteoglycans, hyaluronic acid, GAGs, and collagen. Perlecan is a characteristic component that maintains the structure and character of the PCM.

Perlecan dictates BM stability and integrity in a variety of tissues [20], as PlnIV incorporates into the BM and other extracellular structures via protein–protein interactions [32]. Pericellular colocalization of perlecan COLVI provides matrix stabilization and cell-matrix communication, which allows cells to perceive and respond to perturbations in their biomechanical microenvironment [37]. Further studies revealed that perlecan functions through the COLXI-perlecan-HS connection [38].

Furthermore, perlecan can also result in a significant increase in elasticity [39], whereas deficiency leads to reduced cellular and ECM stiffness in vivo. In addition to its inhibitory effect on COLII synthesis [40], perlecan knockdown also resulted in a decrease in the PCM component and an increase in the bulk matrix, suggesting that the ability of newly synthesized ECM to incorporate into the matrix was impaired without influencing the components of ECM.

Additionally, perlecan and its GAGs impart a fixed negative charge to the ECM, thereby promoting water retention and conferring remarkable shock-absorbing and low-friction properties. When compressed, the interstitial fluid is forced from the tissue to return upon unloading via charge interactions. Concurrently, hydrostatic pressure generated by ECM obstruction of interstitial fluid movement protects the tissue from compressive forces [41].

Osteoarthritic cartilage also exhibited chondron swelling and chondrocyte cluster formation, with pericellular COLIX staining loss, and enlarged chondrons with loosely organized PCM structures. The PCM-specific localization of laminins was reported to be lost in aged, disrupted cartilage, whereas laminin α1 and perlecan were robustly withheld within the PCM in old mice [8], resulting in a loose organization. After trauma and injury, cytokines such as IL-6 disrupt the connection between perlecan and laminins, leading to the loss of perlecan in the PCM and disintegration of the structure. Additionally, OA leads to the release of GAGases and proteases, such as MMPs, the cleavage of HS, and the decline of perlecan, further resulting in the cascade amplification of loss of perlecan [42] and destruction of elasticity and stiffness.

4. Mechanotransduction functions of perlecan on cartilage and chondrocytes

HSPGs modulate the localization, retention, and biological activity of many components in cartilage, and decreased HS content or reduced HS sulfation levels in cartilage are chondroprotective [43]. Increased levels of 6-O-sulphation in OA cartilage contribute to the OA process. 6-O sulfation is a critical modification of HS and controls the interactive properties of many ligands. N-Sulfation of GalNAc by Ndst-1 followed the HS6TI and GLCE biosynthetic steps in the HS assembly. Heterozygous or homozygous Ndst1 mutants may strengthen the ability to preserve the fine structure of PCM due to decreased BMP-2 and -4 activity.

Additionally, removal of perlecan HS from cultured articular chondrocytes promotes cell proliferation and matrix production, suggesting that chondrocytes in mature permanent cartilage do not actively proliferate or lay down matrix components or undergo cartilage expansion because they are surrounded by inhibitory HS sequences in their pericellular perlecan. Deficient cells in HS display accelerated growth, reach hypertrophy earlier, and elaborate ECM PGs to a greater extent than WT mice [44]. Furthermore, heparanase has been shown to accelerate wound angiogenesis and healing in mouse and rat models. Expressed in osteoblastic cells, heparanase also stimulates bone formation and mass [45].

Several growth factors interact with perlecan, are protected from

proteolytic degradation, and are sequestered in the matrix adjacent to cells, where they serve as growth factor reservoirs.

4.1. Through FGF signaling

4.1.1. FGF2

Binding of FGFs-FGFR1 leads to receptor phosphorylation, which in turn activates two critical signaling mediators, Ras and PKCδ. These molecules then integrate their signaling inputs into the Raf-MEK1/2-ERK1/2 cascade, inducing the expression of the transcription factors RUNX2 and ELK1 and accelerating matrix degradation in human articular chondrocytes [46,47] (Fig. 2 A). Disruption of FGFR-1 inhibits the progression of cartilage degeneration [48] in aged mice and in surgically induced mouse models of OA, possibly through downregulation of MMP13 and upregulation of FGFR3 [49].

FGF2 has a high affinity for FGFR1 [50]. Studies have confirmed that FGF2 function is dependent on perlecan [51,52]. FGF2 can bind to PlnI/IV/V which function as a chondrocyte mechanotransductor [53,54]. The data showed that growth plate perlecan binds to FGF-2 by its HS chains but can only deliver FGF-2 to FGF receptors when its CS chains are removed because the CS chain may interdict the connection between HS and the membrane [53] (Fig. 3).

Selective FGFR-ligand interactions drive distinctive signaling pathways, resulting in differential biological outcomes. FGF2 has both deleterious and beneficial effects on the articular cartilage, as it also has an affinity for FGFR3. Apart from the catabolic effects discussed above, other studies have shown that FGF2 exerts anti-catabolic effects on the cartilage in vitro. Specifically, FGF2 can induce Timp1 level in mouse cartilage and suppress IL-1-induced degradative activity [50,55] (Fig. 2 B). However, the affinity of FGF2 for FGFR3 is much weaker than that of FGFR1; hence, FGF2 mainly acts as a pro-catabolic factor.

4.1.2. FGF18

FGF-18 is expressed by normal chondrocytes in the articular cartilage and highly expressed by hypertrophic chondrocytes in chondrocyte columns. FGF18 is a critical anabolic growth factor that is exclusively targeted by FGFR3. FGF18-FGFR3 has also been identified as a signaling molecule that protects articular cartilage from degeneration. Conditional deletion of Fgfr3 in chondrocytes leads to osteoarthritis-like defects in the temporomandibular joint of adult mice. The expression of Indian hedgehog (IHH) was upregulated in the Fgfr3 cKO temporomandibular joint (TMJ), while the primary Fgfr3 cKO chondrocytes were treated with an IHH signaling inhibitor [56–58], indicating that the FGFR3/IHH signaling pathway plays a critical role in maintaining cartilage homeostasis. Studies have also shown that the protective effects of the FGF-18–FGFR3 axis are mediated by the activation of the RAS–MAPK and PI3K–AKT pathways. FGF18 activates PI3K-AKT signal transduction, inhibits apoptosis induced by IL-1β, restores

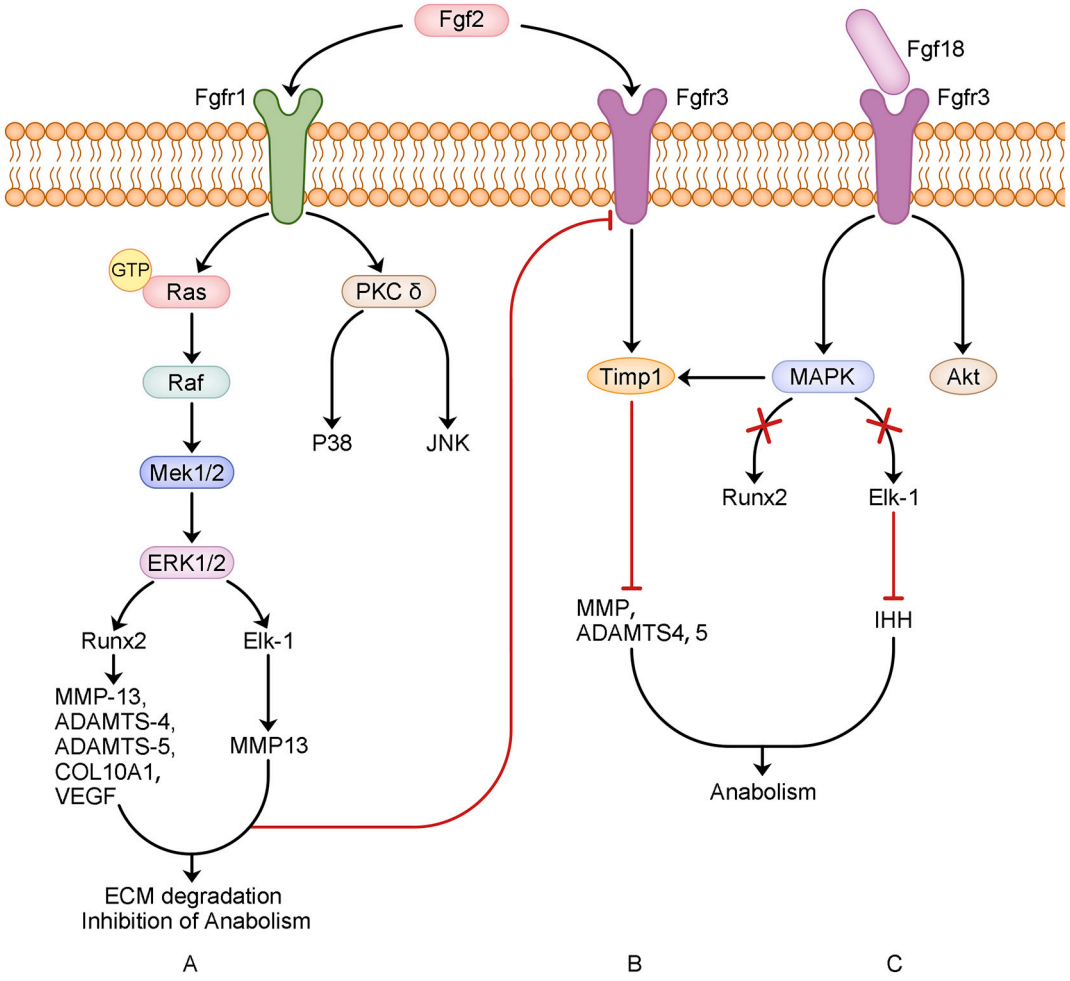

Fig. 2. The regulation pathway of FGFs. (A) The catabolic pathway of FGF2-FGFR1 axis. The binding of FGFs-FGFR1 leads to receptor phosphorylation, which in turn activates Ras and protein kinase C delta (PKCδ). Ras activation triggers Raf-MEK1/2-ERK1/2 cascade, inducing the expression of the transcription factors RUNX2 and ELK1 and accelerating matrix degradation. PKCδ not only plays a synergistic effect with Ras, but also activates p38 and JNK. (B) The anabolic pathway of FGF2-FGFR3 axis. The binding of FGF2-FGFR3 promotes the synthesis of TIMP, which inhibits the function of MMPs. (C) The anabolic pathway of FGF18-FGFR3 axis. The binding of FGF18-FGFR3 activates the RAS–MAPK and PI3K–AKT pathways, the downstream of which is not RUNX2 and ELK1.

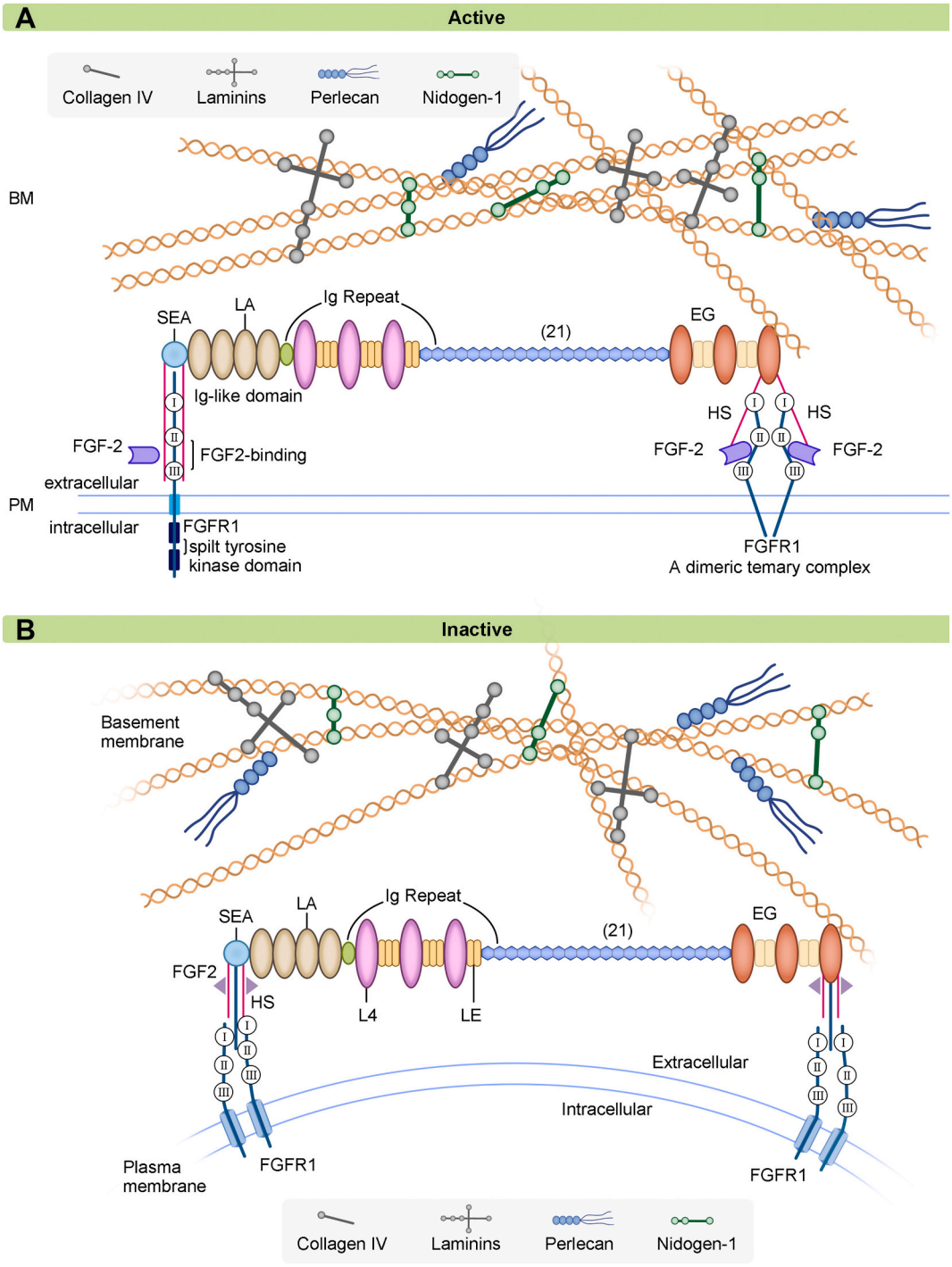

Fig. 3. The different connection patterns of perlecan with adjacent tissues. (A) The connection pattern with both HS and CS chains. (B) Connection pattern with simple HS chains.

mitochondrial function, and reduces the production of reactive oxygen species, demonstrating the anti-OA effect of FGF18 at the cellular level [59]. However, interestingly, activation of MAPK does not lead to downstream access, similar to FGFR1 signaling [46]. FGF-18 inhibits the release and depletion of GAGs from the cartilage by upregulating TIMP1 expression [60], which plays a prominent role in synovial joint development (Fig. 2 C). Meanwhile, FGF18 can stimulate both the proliferation and accumulation of COLII and proteoglycans in adult human articular chondrocytes, chondrogenesis, and cartilage repair by ameliorating the IL-1a–induced Mmp2/Mmp3 increase [61]. The FGF18–FGFR3 axis promotes BMP7-induced cartilage regeneration by suppressing noggin, a natural inhibitor of BMP7 signaling [46].

Further studies showed that FGF18 is connected to the perlecan core protein instead of GAGs to exert its function [31]. Consistent with these findings, HSPG2 IV-3 reduced signal transduction through the ERK pathway. The loss of ERK1/2 phosphorylation was consistent with decreased FoxM1 and Cdkn1c but increased Atf3 mRNA levels [34]. Inhibition of ERK1/2 phosphorylation also prevents cell connections and promotes cell clustering by inhibiting FAK/Src. Meanwhile, HSPG2 IV-3 increases RNA levels associated with mesenchyme condensation and cartilage formation, especially by regulating Sox9 [47]. The mRNA levels of collagen II and aggrecan also increased in the control group

[62]. In general, HSPG2 IV-3, which inactivates ERK signaling and blocks the phosphorylation of FAK and Src [63], may disrupt chondrocyte cell-matrix interactions and cell proliferation, preventing chondrocyte progression to fibrocartilage and cell death from mechanic al load [62]. Thus, perlecan containing FGF18 regulates anabolic and protective progress during the degeneration of chondrocytes, positively regulating cell proliferation and differentiation in osteogenesis and negatively regulating chondrogenesis [64].

There is crosstalk between FGF2-FGFR1 and FGF18-FGFR3, where FGF2 inhibits the expression of FGFR3. Interestingly, the inhibitor of the ERK/MAPK pathway significantly reversed the FGF-2-mediated reduction of FGFR3, suggesting that FGF-2-suppression of FGFR3 also occurs via the FGFR1-ERK/MAPK axis in human articular chondrocytes [50] (Fig. 2 A).

4.2. VEGF

Perlecan in cartilage is required to activate VEGF signaling for vascular invasion and osteoblast migration, which are essential for cartilage matrix remodeling and subsequent bone formation (Fig. 5 C). PlnV (or endorepellin) connects VEGFR2, phosphorylates the Tyr175 target, and modulates the downstream pathway, including the inhibition of degenerative PI3K-AKT-mTOR and activation of PERK-elF2a-ATF4, which leads to angiogenesis [18,65]. LG3 functions may also be important in the pathogenesis of fibrosis by promoting fibroblast survival.

Delta-like ligand4 (Dll4) is a key molecule that contributes to vascular development. VEGF can increase Dll4 level by activating p-ERK1/2, thus facilitating angiogenesis in the TMJ [66] (Fig. 5 D).

4.3. IHh and Wnt

Perlecan transports Wnt and Hh proteins via the LDLR of PlnII [68]. While several Wnt family proteins have been reported to promote degeneration of mature articular cartilage in OA [69–71], 6-O sulfation of perlecan HS chains prevents Wnt protein interactions with its cognate receptor, thus inhibiting Wnt signaling. Desulfation of perlecan HS promotes Wnt signaling and degradative changes in mature articular cartilage, confirming the functional status of perlecan–HS chains [72]. Perlecan transports Wnt in tissues, which are poorly soluble, to establish these concentration gradients [73]. Wnt proteins are palmitoylated signaling glycoproteins that are poorly soluble in aqueous media; however, they bind with a high affinity to PlnII.

4.4. Other signaling

Autophagy has been confirmed as a protective factor against chondrocyte and cartilage [74–76]. Activation of autophagy in chondrocytes by intra-articular injection of resveratrol can significantly delay articular cartilage degeneration in a mouse model of DMM OA [77] and pharmacological suppression or gene deletion of mTOR leads to reduced OA severity [78,79].

Perlecan as a whole inhibits autophagy via mTORC1 activation [80,81]. Mice lacking perlecan expression showed increased autophagy due to inadequate mTORC1 activation in their slow-twitch soleus muscles, as demonstrated by elevated levels of autophagic markers LC3-II and phosphorylated AMPK, alongside decreased phosphorylation of p70S6 K, a downstream target of mTORC1 [82–84] (Figs. 4, 5A).

Previous studies have shown that BMP2 delivered through PlnDI-HA triggers both anabolism by increasing the transcription of proteoglycans and COLII, and protective responses by lowering matrix degradation. Disruption of HS/CS signaling enhances MSC-derived osteogenic differentiation via BMP signaling pathways, and experiments have shown that long-term culture of cells in HS- or CS-degrading enzymes increases bone nodule formation, calcium accumulation, and the expression of alkaline phosphatase, RUNX2, and osteocalcin [85].

5. Changed signal modulated by perlecan in OA

When entering the adult stage, chondrocytes in the articular cartilage stop proliferating and enter a low turnover status to maintain the cartilage extracellular matrix via their anabolic and catabolic actions. Aging, metabolic dysfunction, and mechanical injury can disrupt this balance and lead to progressive cartilage degeneration. Unfortunately, articular cartilage has a limited ability to maintain balance, and with age, these abilities further decline. The degeneration of articular

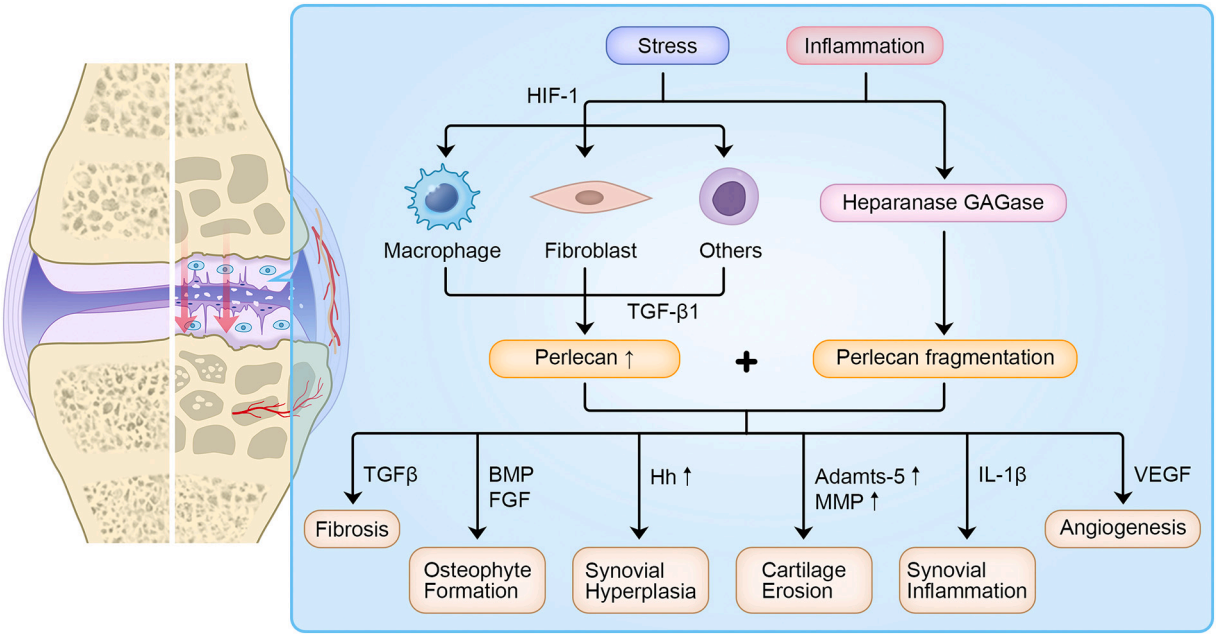

Fig. 4. Changes of articular cartilage in OA. Stress or inflammation factors activate MMP, Runx2, and MTORC1. Dysregulation of these factors induces cartilage degeneration and aberrant bone formation, leading to loss of physical structure and osteophyte formation. Angiogenesis and nerve development may further promote swelling and pain in patients with OA.

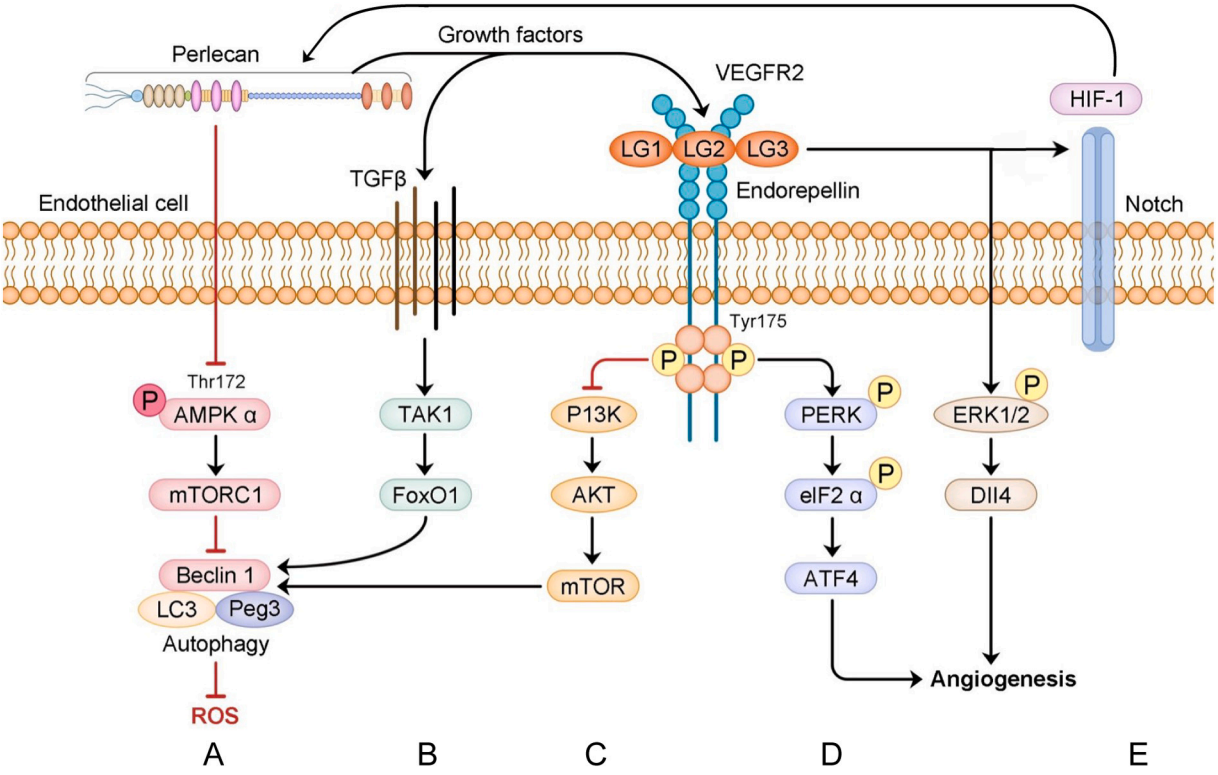

Fig. 5. VEGF-related and other signaling modulated by cartilage perlecan. (A) Perlecan directly enters cells and exerts function, phosphorylates Thr172, and activates the autophagic complex MTORC1 and downstream autophagy related protein. (B) Perlecan binds to the membrane TGFR and activates TAK1, expressing high level FoxO1 and autophagic LC3, Peg3, and Beclin1. (C) Domain V Perlecan (or Endorepellin, part of perlecan) connects VEGFR2, phosphorylates the Tyr175 target, and modulates the downstream pathway, including the inhibition of degenerative PI3K-AKT-mTOR and activation of PERK-eIF2a-ATF4, which leads to angiogenesis. (D) Endorepellin binding to VEGFR2, or binding to Notch which is consequently sheared by MMP, leading to the changes in gene expression, phosphorylation of ERK1/2, activation of Dll4, and further angiogenesis. (E) Inflammatory factors lead to activation and incision of Notch through LG3, inducing HIF-1 pathway and high-level expression of perlecan.

cartilage and associated changes in the synovium and subchondral bone results in joint pain and dysfunction that characterize OA.

Apart from degenerative changes, OA is also characterized by typical inflammation symptoms, such as hyperplasia, including chondrocyte hyperplasia, which leads to the formation of osteophytes and synovium hyperplasia. Clinical manifestations include gradual thickening of the subchondral plate, osteophyte formation, and presence of bone marrow lesions. Lesions and inflammation involve the surrounding connective tissue, which further induces fibrosis. Excessive extracellular matrix and adhesions contract pouches, bursae, and tendons, causing pain and preventing a normal range of joint motion [86] (Fig. 4).

In OA, articular cartilage appears to be hypertrophic, with dysregulated proliferation and survival of chondrocytes, and a loss of collagen, proteoglycans, and cartilage integrity, resulting in impairment of its biomechanical properties [2,87]. Synovitis, a common pathological change in OA, is also a risk factor for severe synovitis that exacerbates cartilage erosion [88]. Proinflammatory factors released by injured joint tissues can induce proliferation and inflammation of the synovium, contributing to synovitis [89] (Fig. 4).

Previous studies have also noticed perlecan alterations in the typical symptoms and revealed the potential role of perlecan in OA and its secondary procedures.

5.1. Perlecan changes in aging induced OA

Studies have demonstrated an inverse correlation between donor age and perlecan expression, as perlecan mRNA levels decrease with donor age [90]. A decline in perlecan can lead to primary hypotrophy [44]. Examination of key autophagy proteins in human and murine cartilage revealed that ULK1, Beclin1, and LC3 protein expression was reduced in human OA chondrocytes and cartilage, but these proteins were strongly expressed in OA cell clusters [91], suggesting that autophagy may be a protective or homeostatic mechanism in normal cartilage, and compromised autophagy represents a novel mechanism in the development of OA. These studies also demonstrate that human aging and OA cartilage and chondrocyte models are associated with a reduction in autophagy and an increase in apoptosis [91].

5.2. Perlecan changes in mechanical damage induced OA

Exercise and injury increase CS length while decreasing HS length in synovial fluid [22], which inhibits perlecan-FGF2-FGFR1 trans-communication, whereas FGF18-FGFR3 would not be affected, as perlecan–FGF-2 signaling is dependent on HS, and FGF-18 can also bind to PlnIII to facilitate receptor activation in an HS-independent manner. Experiments have confirmed that rapid activation of ERK and MAP kinase occurs when articular cartilage is loaded depending upon the release of bFGF, as it is restricted by an FGFR inhibitor, hence inhibiting the synthesis and secretion of TIMP-1 [92].

Meanwhile, another study revealed that perlecan expression was increased in the dynamic loading group [90]. Dynamic loading improves cartilage homeostasis and maintains the chondrogenic phenotype of cartilage. These results suggest that perlecan expression in loaded CTAs is parallel to normal joint function and in vivo pressure, and proper physiological loading maintains perlecan expression in adult cartilage, which contributes to cartilage homeostasis. This partly explains why OA is caused by hyperinactivity.

Inflammatory factors increase VEGF levels. IL-6 activates ERRγ via ERK1/2 [87]. In addition, IL-1β induce the synthesis and release of angiopoietins [87]. All of these have a close affinity for the perlecan HS

chain and interact with perlecan to promote angiogenesis, which is an essential step in the development of OA and the foundation of the formation of osteophytes (Fig. 4).

The degradation of articular cartilage is generally caused by matrix-degrading enzymes activated by cytokines [93]. Fibronectin is upregulated in OA tissues but downregulated in healthy cartilage. Cleaved fibronectin fragments can activate TLR4 signaling, which can increase the expression of inflammatory cytokines and upregulate MMPs. MMP3 targeted at GAGs on perlecan causes fragmentation and further development of OA [94]. Purified perlecan shows a high sensitivity to the digestion of MMP-7, even when fully decorated with HS or linked to other BM proteins in the natural environment. Under both conditions, MMP-7 produced discrete perlecan fragments corresponding to Ig repeats on PlnIV [95].

During wound healing, the GAG chains of perlecan are cleaved by GAGases, releasing HBGFs directly at the site of injury [16], where FGF2 interacts with FGFR1 on the chondrocyte membrane.

Cleavage leads to the fragmentation of perlecan, which may exert an opposite effect on cells, leading to catabolic actions such as inhibition of proteoglycan accumulation and induction of cell proliferation. The levels of perlecan protein and mRNA were upregulated, especially in elongated secretory type 2 cells in the area adjacent to the main cartilage defect. This might be seen as an attempt at stabilizing the extracellular matrix in part of the cartilage tissue. Even though a study revealed that perlecan expression was increased in the dynamic loading group [90], OA articular cartilage demonstrated loss of perlecan [39], indicating that OA impaired the ability of newly synthesized perlecan to incorporate into the matrix bulk.

5.3. Universal perlecan changes in OA

Fibrosis, a typical secondary response to inflammatory hyperplasia, is common in advanced OA. The final stage of fibrosis development is characterized by a significant change in the structure of the ECM due to increased synthesis and decreased proteolytic turnover. The increased production of ECM by cells, including collagen types I and III, perlecan, and laminins, causes a net buildup of ECM in wounded tissues, which promotes fibrosis. We discussed that perlecan can interact with many cytokines through HS. These factors promote the migration of fibrogenic cells to the site of injury thereby amplifying inflammation and fibrous [96,97].

Previous studies have shown the reverse correlation between the osteophyte size or maturation and the HS chains in the surgical OA model, suggesting that the reduced osteophyte formation in Hspg2$^{-/-}$-Tg mice was associated with reduced cell proliferation and chondrogenesis synovial perlecan plays an important role in osteophyte development in OA. Quantitative analysis confirmed the significant reduction of p-Smad2 in the osteophytes of Hspg2$^{-/-}$-Tg mice, indicating the necessity of OA development via TGF-β/Smad signaling [98]. Consistent with the reduced level of perlecan in the elderly, cartilage showed a highly reduced capacity for mechanically mediated activation of Smad2/3P signaling during aging [99].

Additionally, studies have shown that stimulation of cartilage tissue with IL-1a significantly inhibited Fgfr1 expression to the same extent in both genotypes. In contrast, IL-1a decreased the expression of Fgfr3 to a significantly greater extent in WT mice than in Hspg2$^{D32/D32}$ mice. These findings suggest that perlecan HS plays a significant role in directing the progression of posttraumatic OA by altering joint inflammation and cartilage degradation, and regulating pathways that promote osteophyte development. The effects of the loss of perlecan HS in cartilage appear to be mediated, in part, by reducing FGF matrix sequestration and MMP3 levels and by preserving FGFR-3 [61].

Generally, HSPG plays an important role in modulating the localization, retention, and biological activity of many components of cartilage [100]. It is involved in the modulation of synovial fibrosis, chondrogenic differentiation through the transmission of BMP and FGF, hypertrophy and ossification through Hh signaling, matrix degradation through cleavage of MMP13 and Adamts-5, inflammation through the adjustment of IL- 1β and subchondral bone formation through TGF-β or Wnt/b-catenin. There is also weak evidence that perlecan mediates pain in OA through β-NGF-related pathways [101].

Perlecan expression is upregulated and dysregulated in hypertrophic growth plate chondrocytes and OA [100], possibly because cells that infiltrate the injured site during inflammation and repair, including circulating inflammatory cells and fibrocytes, contribute to perlecan synthesis. Perlecan can be produced by macrophages, granulocytes, and monocyte-derived macrophages in response to hypoxia, mediated by the HIF-1 pathway. Cytokines such as TGFβ1, which is expressed by many cell types involved in fibrogenesis, can also upregulate perlecan by activating plasmin [102] (Fig. 5 E).

Heparanase in OA cartilage induces catabolic responses [103] but also downregulates anabolic genes. Reduced HS levels, reduced HS sulfation, and alterations in HS fine structure were observed in OA cartilage, which had decreased degenerative OA histopathology scores [43].

However, it is only when HS is degraded or perlecan undergoes proteolytic degradation by MMP-13 at the chondro-osseous junction where bone formation occurs. Osteoblasts synthesize abundant levels of heparanase, and hypertrophic chondrocytes of the chondro-osseous junction contain abundant levels of MMP-13, which degrades perlecan in this region, releasing HS and core protein from PlnI. The elevated production of heparanase by osteoblasts and degradation of perlecan–HS releases bound growth factors that stimulate angiogenesis and promote bone formation [18,103–105].

While the HS chains of perlecan play indispensable roles in embryogenesis and skeletogenesis, they can also inhibit adult tissue repair processes. Degradation of HS in situ improves wound healing and tissue repair through the re-mobilization of previously sequestered FGF-2, which was unavailable to promote tissue repair, as the results demonstrated that heparanase releases FGF-2 in tissues in an activated form, which promotes wound healing [45,103].

6. Treatment prospects of perlecan in OA

Given the role of perlecan in OA, perlecan has also been considered as a therapeutic target for repair [106].

As discussed above, load and exercise can increase the level of perlecan and the length of CS, thereby promoting the protective action of the articular cartilage. Previous randomized controlled clinical trials (RCTs) have proven the efficacy of manual physical therapy in yielding functional benefits and delaying or preventing surgical intervention [107]. Further meta-analysis has also confirmed these results [108]. The effects of multiple exercise patterns have also been compared, including resistance training [109], aquatic [110], and land-based, all of which have been found to benefit patients' symptoms and prognosis. Self-management of exercise has been included in the recent guidelines for OA [111]. However, further studies focusing on the relationship between exercise and perlecan are lacking. Whether the benefits of exercise function, at least partially, through perlecan, deserve discussion and research.

The high fixed charge density and counter ions of GAGs are central to their roles in the hydration of various connective tissues within the body. The electro-conductive properties of GAGs containing these functional groups that transfer electric charge and modulate cellular activity can be modified by electronic stimulation [112]. Thus, electrostimulation has been proposed as a potential therapeutic treatment through RCT [113], and a recent meta-analysis confirmed its efficacy [114] and promoted its transfer to clinical practice.

Apart from the physical therapy, pharmacological treatment is another potential treatment pattern.

The first version of pharmacological treatment uses perlecan as a drug delivery tool. A large number of new drugs have been developed

based on traditional drugs. Intra-articular (IA) administration for OA has hastened the development of targeted drug delivery systems (DDS). OA drug modification and the synthesis of bioadaptive carriers contribute to a qualitative leap in the efficacy of IA treatment. Perlecan, which has important matrix stabilizing properties and sequesters, controls, and presents growth factors to cellular receptors, can act as a carrier of some drugs and regulatory factors, inducing them to function. For example, injectable perlecan domain 1-hyaluronan microgels potentiate the cartilage repair effect of BMP2 in a murine model of early osteoarthritis [115]. Antibodies targeted at known signaling pathways are also effective in inhibiting OA; for example, bevacizumab, an anti-vascular EGF antibody, showed positive results in in vitro and animal studies; however, clinical trials are needed to confirm the positive impact on cartilage repair [116,117]. PPS, a semi-synthetic heparinoid DMOAD, can be combined with the perlecan core protein, offering new opportunities in repair biology [118]. Although preclinical studies are still being conducted, perlecan is likely to be effective as a drug delivery system [119].

Another version is the application of the Perlecan transmission effect. In accordance with the role of perlecan in OA, we can control its level or structure and target it downstream to inhibit injury.

Given the multiple factors balanced by perlecan, we can target them by administering recombinant factors, specific inhibitors, and antibodies. For example, sprifermin, a type of recombinant FGF-18, has been investigated as a disease-modifying osteoarthritis drug. In a 5-years lasting RCT, we noticed a statistically significant improvement in total femorotibial joint cartilage thickness in a high-dose (intra-articular injections of 100 μg every 6 months or 12 months) sprifermin-administered population [120]. Sprifermin sustained long-term structural alteration of articular cartilage over 3.5 years post-treatment in the longest DMOAD experiment reported to date. SAR has revealed potential therapeutic benefits that might be translated [121]. It is noteworthy that the protective effect of sprifermin is independent of its location [122]. There are also many other potential therapeutics such as IL-1β receptor antagonists [123,124], monoclonal antibodies against β-NGF (tanezumab) [125], Smo inhibitors, and MMP13 inhibitors [126]. Current clinical experiments have shown positive but no apparent adverse effects and require further RCT [101,127].

Proteoglycan degradation adversely affects the functional properties of tissues. Therefore, therapeutic strategies have been developed to counter these degenerative changes [128]. Given the important role of the subside chains of perlecan, some studies have focused on perlecan modifications, such as injection or oral chondroitin [129], which have been proven to be effective in RCT [130]. Low-dose, short-term acetaminophen, pharmaceutical grade glucosamine, and chondroitin sulfate are recommended by ESCEO, whereas OARSI strongly recommends against their use in the 2019 guidelines [131]. Other studies have also confirmed that the cleavage and recombination of specific domains or GAGs interacting with specific molecules [49] and structural modification of the sulfate substrate [43] can cut off the process of OA. Heparanase treatment of chondrocytes in vitro resulted in significantly increased proliferation and increased chondrocyte responsiveness to cartilage injury, and perhaps improved repair of defects [106]. Further studies should be conducted to ensure the related feasibility, efficacy, and safety in clinical practice.

There are other problems that deserve further discussion. In previous studies, we noticed contradictory roles for FGF2 in articular cartilage homeostasis. However, the reasons for this are unclear. However, some studies have noticed two different types of FGF2, high-molecular-weight FGF2 isoforms (HMWTg) and low-molecular-weight FGF2 isoforms (LMWTg), which have diverse effects. HMWTg mice spontaneously develop signs of OA as early as 2 months of age, whereas LMWTg mice are protected from OA [132,133]. Moreover, LMWKO OA cartilage exhibited increased FGF2, FGF23, and FGFR1 expression, whereas HMWKO cartilage exhibited increased levels of FGFR3 [132]. Based on this information, we hypothesized that the contradictory roles of FGF2 in articular cartilage homeostasis are attributable to the different types of FGF2 exclusively targeted at different receptors. Further verification of this hypothesis is needed.

7. Conclusion

In conclusion, perlecan has diverse effects during different stages. In normal cartilage, it maintains the integrin of the PCM and sequesters growth factors. After trauma, the perlecan expression first upregulates as a compensatory mechanism, but as a result of the loss of integrin and the increased consumption, the perlecan level soon downregulates, leading to the destruction of the normal structure. During the post-trauma period, it is also a key molecule that promotes the development of OA, especially contributing to chondrocyte hypotrophy, angiogenesis, osteophyte formation, synovium fibrosis, and autophagy regulation between chondrocytes and PCM. Perlecan effectively fits the gap between risk factors and downstream OA outcomes, and has profound treatment prospects.

Abbreviations

OA	Osteoarthritis
PCM	pericellular matrix
HS	heparan sulfate
CS	chondroitin sulfate
GAG	glycosaminoglycan
SHH	sonic hedgehog
WARP	Willebrand factor A domain related protein
IHH	Indian hedgehog
TMJ	temporomandibular joint
RCT	randomized, controlled clinical trial

CRediT authorship contribution statement

Xiao-Xuan Zhao and Wen-Qing Xie wrote the original draft. Wen-Feng Xiao, Heng-Zhen Li and Shinen Naranmandakh wrote and edited the review. Wen-Qing Xie, Olivier Bruyere and Xiao-Xuan Zhao prepared the figures. Jean-Yves Reginster and Yu-Sheng Li edited the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declared no conflict of interest.

Acknowledgments

This work was supported by National Key R&D Program of China (2019YFA0111900), National Natural Science Foundation of China (81874030, 82072506), Science and Technology Innovation Program of Hunan Province (2021RC3025), Provincial Clinical Medical Technology Innovation Project of Hunan (2020SK53709), InnovationDriven Project of Central South university (2020CX045), Wu Jieping Medical Foundation (320.6750.2020-03-14), the Independent Exploration and Innovation Project for Postgraduate Students of Central South University (2021zzts1024) and the Hunan Provincial Innovation Foundation for Postgraduate (CX20210360).

References

[1] S. Glyn-Jones, et al., Osteoarthritis, Lancet 386 (9991) (2015) 376–387.
[2] J. Martel-Pelletier, Osteoarthritis, Nat. Rev. Dis. Primers. 2 (2016) 16072.
[3] D.B. Burr, M.A. Gallant, Bone remodelling in osteoarthritis, Nat. Rev. Rheumatol. 8 (11) (2012) 665–673.
[4] C. Liu, et al., Protective effects of the pericellular matrix of chondrocyte on articular cartilage against the development of osteoarthritis, Histol. Histopathol. 33 (8) (2018) 757–764.
[5] S.J. Gilbert, C.S. Bonnet, E.J. Blain, Mechanical cues: bidirectional reciprocity in the extracellular matrix drives mechano-signalling in articular cartilage, Int. J. Mol. Sci. 22 (24) (2021).

[6] S.C. Sibole, et al., The protective function of directed asymmetry in the pericellular matrix enveloping chondrocytes, Ann. Biomed. Eng. 50 (1) (2022) 39–55.
[7] R.E. Wilusz, J. Sanchez-Adams, F. Guilak, The structure and function of the pericellular matrix of articular cartilage, Matrix Biol. 39 (2014) 25–32.
[8] F. Guilak, et al., Osteoarthritis as a disease of the cartilage pericellular matrix, Matrix Biol. 71–72 (2018) 40–50.
[9] D.R. Chery, et al., Early changes in cartilage pericellular matrix micromechanobiology portend the onset of post-traumatic osteoarthritis, Acta Biomater. 111 (2020) 267–278.
[10] A.D. Snow, et al., Differential binding of vascular cell-derived proteoglycans (perlecan, biglycan, decorin, and versican) to the beta-amyloid protein of Alzheimer's disease, Arch. Biochem. Biophys. 320 (1) (1995) 84–95.
[11] P.Y. Rescan, et al., Distribution and origin of the basement membrane component perlecan in rat liver and primary hepatocyte culture, Am. J. Pathol. 142 (1) (1993) 199–208.
[12] J. Melrose, et al., The structure, location, and function of perlecan, a prominent pericellular proteoglycan of fetal, postnatal, and mature hyaline cartilages, J. Biol. Chem. 281 (48) (2006) 36905–36914.
[13] J. Melrose, Perlecan, a modular instructive proteoglycan with diverse functional properties, Int. J. Biochem. Cell Biol. 128 (2020), 105849.
[14] P.Y. Lin, et al., A novel pathogenic HSPG2 mutation in schwartz-jampel syndrome, Front. Neurol. 12 (2021), 632336.
[15] G. Gao, et al., Impact of perlecan, a core component of basement membrane, on regeneration of cartilaginous tissues, Acta Biomater. 135 (2021) 13–26.
[16] J.R. Martinez, A. Dhawan, M.C. Farach-Carson, Modular proteoglycan Perlecan/HSPG2: mutations, phenotypes, and functions, Genes (Basel) 9 (11) (2018).
[17] M.C. Farach-Carson, D.D. Carson, Perlecan–a multifunctional extracellular proteoglycan scaffold, Glycobiology 17 (9) (2007) 897–905.
[18] M. Ishijima, et al., Perlecan modulates VEGF signaling and is essential for vascularization in endochondral bone formation, Matrix Biol. 31 (4) (2012) 234–245.
[19] A.D. Murdoch, et al., Primary structure of the human heparan sulfate proteoglycan from basement membrane (HSPG2/perlecan). A chimeric molecule with multiple domains homologous to the low density lipoprotein receptor, laminin, neural cell adhesion molecules, and epidermal growth factor, J. Biol. Chem. 267 (12) (1992) 8544–8557.
[20] R.V. Iozzo, Basement membrane proteoglycans: from cellar to ceiling, Nat. Rev. Mol. Cell Biol. 6 (8) (2005) 646–656.
[21] P. Bork, L. Patthy, The SEA module: a new extracellular domain associated with O-glycosylation, Protein Sci. 4 (7) (1995) 1421–1425.
[22] M.P. Brown, et al., Exercise and injury increase chondroitin sulfate chain length and decrease hyaluronan chain length in synovial fluid, Osteoarthr. Cartil. 15 (11) (2007) 1318–1325.
[23] W.D. Yang, et al., Perlecan domain I promotes fibroblast growth factor 2 delivery in collagen I fibril scaffolds, Tissue Eng. 11 (1–2) (2005) 76–89.
[24] C.Y. Chuang, et al., Heparan sulfate-dependent signaling of fibroblast growth factor 18 by chondrocyte-derived perlecan, Biochemistry 49 (26) (2010) 5524–5532.
[25] Y. Xu, Y.-J. Liu, Q. Yu, Angiopoietin-3 is tethered on the cell surface via heparan sulfate proteoglycans *, J. Biol. Chem. 279 (39) (2004) 41179–41188.
[26] M. Akerlund, et al., Laminin alpha1 domains LG4-5 are essential for the complete differentiation of visceral endoderm, Cell Tissue Res. 338 (1) (2009) 129–137.
[27] E. Bengtsson, et al., The leucine-rich repeat protein PRELP binds perlecan and collagens and may function as a basement membrane Anchor*, J. Biol. Chem. 277 (17) (2002) 15061–15068.
[28] J.C. Brown, et al., The C-terminal domain V of perlecan promotes β1 integrin-mediated cell adhesion, binds heparin, nidogen and Fibulin-2 and can be modified by glycosaminoglycans, Eur. J. Biochem. 250 (1) (1997) 39–46.
[29] T. Nishida, et al., CTGF/Hcs24, hypertrophic chondrocyte-specific gene product, interacts with perlecan in regulating the proliferation and differentiation of chondrocytes, J. Cell. Physiol. 196 (2) (2003) 265–275.
[30] K. Tiedemann, et al., Microfibrils at basement membrane zones interact with perlecan via fibrillin-1, J. Biol. Chem. 280 (12) (2005) 11404–11412.
[31] S.M. Smith, L.A. West, J.R. Hassell, The core protein of growth plate perlecan binds FGF-18 and alters its mitogenic effect on chondrocytes, Arch. Biochem. Biophys. 468 (2) (2007) 244–251.
[32] M. Hopf, et al., Recombinant domain IV of perlecan binds to nidogens, laminin-nidogen complex, fibronectin, fibulin-2 and heparin, Eur. J. Biochem. 259 (3) (1999) 917–925.
[33] M. Hopf, et al., Mapping of binding sites for nidogens, fibulin-2, fibronectin and heparin to different IG modules of perlecan, J. Mol. Biol. 311 (3) (2001) 529–541.
[34] J.R. Martinez, et al., Perlecan/HSPG2: signaling role of domain IV in chondrocyte clustering with implications for schwartz-jampel syndrome, J. Cell. Biochem. 120 (2) (2019) 2138–2150.
[35] W. Göhring, et al., Mapping of the binding of platelet-derived growth factor to distinct domains of the basement membrane proteins BM-40 and perlecan and distinction from the BM-40 collagen-binding epitope, Eur. J. Biochem. 255 (1) (1998) 60–66.
[36] M. Mongiat, et al., Perlecan protein core interacts with extracellular matrix protein 1 (ECM1), a glycoprotein involved in bone formation and angiogenesis, J. Biol. Chem. 278 (19) (2003) 17491–17499.
[37] A.J. Hayes, et al., Pericellular colocalisation and interactive properties of type VI collagen and perlecan in the intervertebral disc, Eur. Cell. Mater. 32 (2016) 40–57.
[38] S.M. Smith, J. Melrose, Type XI collagen-perlecan-HS interactions stabilise the pericellular matrix of annulus fibrosus cells and chondrocytes providing matrix stabilisation and homeostasis, J. Mol. Histol. 50 (3) (2019) 285–294.
[39] R.E. Wilusz, L.E. Defrate, F. Guilak, A biomechanical role for perlecan in the pericellular matrix of articular cartilage, Matrix Biol. 31 (6) (2012) 320–327.
[40] R. Nakamura, F. Nakamura, S. Fukunaga, Disruption of endogenous perlecan function improves differentiation of rat articular chondrocytes in vitro, Anim. Sci. J. 86 (4) (2015) 449–458.
[41] O.R. Schätti, et al., Mechanical loading of cartilage explants with compression and sliding motion modulates gene expression of lubricin and catabolic enzymes, Cartilage 6 (3) (2015) 185–193.
[42] A.R. Ocken, et al., Perlecan knockdown significantly alters extracellular matrix composition and organization during cartilage development, Mol. Cell. Proteomics 19 (7) (2020) 1220–1235.
[43] A.C. Severmann, et al., An altered heparan sulfate structure in the articular cartilage protects against osteoarthritis, Osteoarthr. Cartil. 28 (7) (2020) 977–987.
[44] C.C. Shu, et al., Elevated hypertrophy, growth plate maturation, glycosaminoglycan deposition, and exostosis formation in the Hspg2 exon 3 null mouse intervertebral disc, Biochem. J. 476 (2) (2019) 225–243.
[45] E. Zcharia, et al., Heparanase accelerates wound angiogenesis and wound healing in mouse and rat models, FASEB J. 19 (2) (2005) 211–221.
[46] M.B. Ellman, et al., Fibroblast growth factor control of cartilage homeostasis, J. Cell. Biochem. 114 (4) (2013) 735–742.
[47] J.N. Janssen, et al., The influence of TGF-β3, EGF, and BGN on SOX9 and RUNX2 expression in human chondrogenic progenitor cells, J. Histochem. Cytochem. 67 (2) (2019) 117–127.
[48] T. Weng, et al., Genetic inhibition of fibroblast growth factor receptor 1 in knee cartilage attenuates the degeneration of articular cartilage in adult mice, Arthritis Rheum. 64 (12) (2012) 3982–3992.
[49] Q. Tan, et al., A novel FGFR1-binding peptide attenuates the degeneration of articular cartilage in adult mice, Osteoarthr. Cartil. 26 (12) (2018) 1733–1743.
[50] D. Yan, et al., Fibroblast growth factor receptor 1 is principally responsible for fibroblast growth factor 2-induced catabolic activities in human articular chondrocytes, Arthritis Res. Ther. 13 (4) (2011), R130.
[51] C. Sun, et al., Selectivity in glycosaminoglycan binding dictates the distribution and diffusion of fibroblast growth factors in the pericellular matrix, Open Biol. 6 (3) (2016).
[52] T. Hodgkinson, et al., Mechanosignalling in cartilage: an emerging target for the treatment of osteoarthritis, Nat. Rev. Rheumatol. 18 (2) (2022) 67–84.
[53] S.M. Smith, et al., Heparan and chondroitin sulfate on growth plate perlecan mediate binding and delivery of FGF-2 to FGF receptors, Matrix Biol. 26 (3) (2007) 175–184.
[54] Z. Zhao, et al., Mechanotransduction pathways in the regulation of cartilage chondrocyte homoeostasis, J. Cell. Mol. Med. 24 (10) (2020) 5408–5419.
[55] Y. Xie, et al., Fibroblast growth factor signalling in osteoarthritis and cartilage repair, Nat. Rev. Rheumatol. 16 (10) (2020) 547–564.
[56] J. Tang, et al., Fibroblast growth factor receptor 3 inhibits osteoarthritis progression in the knee joints of adult mice, Arthritis Rheumatol. 68 (10) (2016) 2432–2443.
[57] S. Zhou, et al., Conditional deletion of Fgfr3 in chondrocytes leads to osteoarthritis-like defects in temporomandibular joint of adult mice, Sci. Rep. 6 (2016) 24039.
[58] S. Woods, et al., miR-324-5p is up regulated in end-stage osteoarthritis and regulates indian hedgehog signalling by differing mechanisms in human and mouse, Matrix Biol. 77 (2019) 87–100.
[59] X. Yao, et al., Fibroblast growth factor 18 exerts anti-osteoarthritic effects through PI3K-AKT signaling and mitochondrial fusion and fission, Pharmacol. Res. 139 (2019) 314–324.
[60] Y. Mori, et al., Identification of fibroblast growth factor-18 as a molecule to protect adult articular cartilage by gene expression profiling, J. Biol. Chem. 289 (14) (2014) 10192–10200.
[61] C.C. Shu, et al., Ablation of perlecan domain 1 heparan sulfate reduces progressive cartilage degradation, synovitis, and osteophyte size in a preclinical model of posttraumatic osteoarthritis, Arthritis Rheumatol. 68 (4) (2016) 868–879.
[62] R. Sadatsuki, et al., Perlecan is required for the chondrogenic differentiation of synovial mesenchymal cells through regulation of Sox9 gene expression, J. Orthop. Res. 35 (4) (2017) 837–846.
[63] I. Hunger-Glaser, et al., PDGF and FGF induce focal adhesion kinase (FAK) phosphorylation at Ser-910: dissociation from Tyr-397 phosphorylation and requirement for ERK activation, J. Cell. Physiol. 200 (2) (2004) 213–222.
[64] N. Ohbayashi, et al., FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis, Genes Dev. 16 (7) (2002) 870–879.
[65] J.J. Zoeller, J.M. Whitelock, R.V. Iozzo, Perlecan regulates developmental angiogenesis by modulating the VEGF-VEGFR2 axis, Matrix Biol. 28 (5) (2009) 284–291.
[66] Y. Dong, et al., VEGF promotes cartilage angiogenesis by phospho-ERK1/2 activation of Dll4 signaling in temporomandibular joint osteoarthritis caused by chronic sleep disturbance in wistar rats, Oncotarget 8 (11) (2017) 17849–17861.
[67] Y. Chen, et al., HIF-1-VEGF-notch mediates angiogenesis in temporomandibular joint osteoarthritis, Am. J. Transl. Res. 11 (5) (2019) 2969–2982.
[68] L. Pulsatelli, et al., New findings in osteoarthritis pathogenesis: therapeutic implications, Ther. Adv. Chronic Dis. 4 (1) (2013) 23–43.

[69] M. De Santis, et al., The role of wnt pathway in the pathogenesis of OA and its potential therapeutic implications in the field of regenerative medicine, Biomed. Res. Int. 2018 (2018), 7402947.
[70] R.J. Lories, S. Monteagudo, Review article: is wnt signaling an attractive target for the treatment of osteoarthritis? Rheumatol. Ther. 7 (2) (2020) 259–270.
[71] W.F. Xiao, et al., Functional role of hedgehog pathway in osteoarthritis, Cell Biochem. Funct. 38 (2) (2020) 122–129.
[72] X. Ai, et al., QSulf1 remodels the 6-O sulfation states of cell surface heparan sulfate proteoglycans to promote wnt signaling, J. Cell Biol. 162 (2) (2003) 341–351.
[73] I. Matsuo, C. Kimura-Yoshida, Extracellular distribution of diffusible growth factors controlled by heparan sulfate proteoglycans during mammalian embryogenesis, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 369 (1657) (2014).
[74] R. Duan, H. Xie, Z.Z. Liu, The role of autophagy in osteoarthritis, Front. Cell Dev. Biol. 8 (2020), 608388.
[75] G. Musumeci, et al., Biomarkers of chondrocyte apoptosis and autophagy in osteoarthritis, Int. J. Mol. Sci. 16 (9) (2015) 20560–20575.
[76] S. Wang, et al., The role of autophagy and mitophagy in bone metabolic disorders, Int. J. Biol. Sci. 16 (14) (2020) 2675–2691.
[77] N. Qin, et al., Local intra-articular injection of resveratrol delays cartilage degeneration in C57BL/6 mice by inducing autophagy via AMPK/mTOR pathway, J. Pharmacol. Sci. 134 (3) (2017) 166–174.
[78] K. Takayama, et al., Local intra-articular injection of rapamycin delays articular cartilage degeneration in a murine model of osteoarthritis, Arthritis Res. Ther. 16 (6) (2014) 482.
[79] M. Ribeiro, et al., Diabetes-accelerated experimental osteoarthritis is prevented by autophagy activation, Osteoarthr. Cartil. 24 (12) (2016) 2116–2125.
[80] B. Caramés, et al., Autophagy activation by rapamycin reduces severity of experimental osteoarthritis, Ann. Rheum. Dis. 71 (4) (2012) 575–581.
[81] B. Caramés, et al., The relationship of autophagy defects to cartilage damage during joint aging in a mouse model, Arthritis Rheumatol. 67 (6) (2015) 1568–1576.
[82] L. Ning, et al., Perlecan inhibits autophagy to maintain muscle homeostasis in mouse soleus muscle, Matrix Biol. 48 (2015) 26–35.
[83] T. Neill, et al., A functional outside-in signaling network of proteoglycans and matrix molecules regulating autophagy, Matrix Biol. 100–101 (2021) 118–149.
[84] C.G. Chen, R.V. Iozzo, Extracellular matrix guidance of autophagy: a mechanism regulating cancer growth, Open Biol. 12 (1) (2022), 210304.
[85] K.J. Manton, et al., Disruption of heparan and chondroitin sulfate signaling enhances mesenchymal stem cell-derived osteogenic differentiation via bone morphogenetic protein signaling pathways, Stem Cells 25 (11) (2007) 2845–2854.
[86] K.M. Usher, et al., Pathological mechanisms and therapeutic outlooks for arthrofibrosis, Bone Res. 7 (2019) 9.
[87] B. Li, et al., Pathological mechanism of chondrocytes and the surrounding environment during osteoarthritis of temporomandibular joint, J. Cell. Mol. Med. 25 (11) (2021) 4902–4911.
[88] F.W. Roemer, et al., Presence of MRI-detected joint effusion and synovitis increases the risk of cartilage loss in knees without osteoarthritis at 30-month follow-up: the MOST study, Ann. Rheum. Dis. 70 (10) (2011) 1804–1809.
[89] R. Liu-Bryan, R. Terkeltaub, Emerging regulators of the inflammatory process in osteoarthritis, Nat. Rev. Rheumatol. 11 (1) (2015) 35–44.
[90] A. van Caam, et al., Perlecan expression is strongly reduced in aging cartilage but increased by physiological loading, Osteoarthr. Cartil. 22 (2014), S60.
[91] B. Caramés, et al., Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis, Arthritis Rheum. 62 (3) (2010) 791–801.
[92] T.L. Vincent, et al., Basic fibroblast growth factor mediates transduction of mechanical signals when articular cartilage is loaded, Arthritis Rheum. 50 (2) (2004) 526–533.
[93] M.B. Goldring, M. Otero, Inflammation in osteoarthritis, Curr. Opin. Rheumatol. 23 (5) (2011) 471–478.
[94] P.K. Sacitharan, Ageing and osteoarthritis, Subcell. Biochem. 91 (2019) 123–159.
[95] B.J. Grindel, et al., Matrilysin/MMP-7 cleavage of Perlecan/HSPG2 complexed with semaphorin 3A supports FAK-mediated stromal invasion by prostate cancer cells, Sci. Rep. 8 (1) (2018) 7262.
[96] H. Sahin, C. Trautwein, H.E. Wasmuth, Functional role of chemokines in liver disease models, Nat. Rev. Gastroenterol. Hepatol. 7 (12) (2010) 682–690.
[97] P. Saikia, et al., Basement membranes in the cornea and other organs that commonly develop fibrosis, Cell Tissue Res. 374 (3) (2018) 439–453.
[98] H. Kaneko, et al., Synovial perlecan is required for osteophyte formation in knee osteoarthritis, Matrix Biol. 32 (3–4) (2013) 178–187.
[99] W. Madej, et al., Ageing is associated with reduction of mechanically-induced activation of Smad2/3P signaling in articular cartilage, Osteoarthr. Cartil. 24 (1) (2016) 146–157.
[100] A. Chanalaris, et al., Heparan sulfate proteoglycan synthesis is dysregulated in human osteoarthritic cartilage, Am. J. Pathol. 189 (3) (2019) 632–647.
[101] W. Zhang, et al., Current research on pharmacologic and regenerative therapies for osteoarthritis, Bone Res. 4 (2016) 15040.
[102] M.S. Lord, et al., The multifaceted roles of perlecan in fibrosis, Matrix Biol. 68–69 (2018) 150–166.
[103] A.J. Hayes, J. Whitelock, J. Melrose, Regulation of FGF-2, FGF-18 and transcription factor activity by perlecan in the maturational development of transitional rudiment and growth plate cartilages and in the maintenance of permanent cartilage homeostasis, Int. J. Mol. Sci. 23 (4) (2022).
[104] D.J. Behonick, et al., Role of matrix metalloproteinase 13 in both endochondral and intramembranous ossification during skeletal regeneration, PLoS One 2 (11) (2007), e1150.
[105] S.E. Usmani, et al., Transforming growth factor alpha controls the transition from hypertrophic cartilage to bone during endochondral bone growth, Bone 51 (1) (2012) 131–141.
[106] J. Garcia, et al., Perlecan in the natural and cell therapy repair of human adult articular cartilage: can modifications in this proteoglycan be a novel therapeutic approach? Biomolecules 11 (1) (2021).
[107] G.D. Deyle, et al., Effectiveness of manual physical therapy and exercise in osteoarthritis of the knee. A randomized, controlled trial, Ann. Intern. Med. 132 (3) (2000) 173–181.
[108] S.L. Goh, et al., Efficacy and potential determinants of exercise therapy in knee and hip osteoarthritis: a systematic review and meta-analysis, Ann. Phys. Rehabil. Med. 62 (5) (2019) 356–365.
[109] K.R. Vincent, et al., Eccentric and concentric resistance exercise comparison for knee osteoarthritis, Med. Sci. Sports Exerc. 51 (10) (2019) 1977–1986.
[110] H.P. French, J.H. Abbott, R. Galvin, Adjunctive therapies in addition to land-based exercise therapy for osteoarthritis of the hip or knee, Cochrane Database Syst. Rev. 10 (10) (2022), Cd011915.
[111] C. Gay, Educating patients about the benefits of physical activity and exercise for their hip and knee osteoarthritis. Systematic literature review, Ann. Phys. Rehabil. Med. 59 (3) (2016) 174–183.
[112] A.J. Hayes, J. Melrose, Electro-stimulation, a promising therapeutic treatment modality for tissue repair: emerging roles of sulfated glycosaminoglycans as electro-regulatory mediators of intrinsic repair processes, Adv. Ther. 3 (11) (2020), 2000151.
[113] S. Ahsin, et al., Clinical and endocrinological changes after electro-acupuncture treatment in patients with osteoarthritis of the knee, Pain 147 (1–3) (2009) 60–66.
[114] N. Chen, et al., Electro-acupuncture is beneficial for knee osteoarthritis: the evidence from meta-analysis of randomized controlled trials, Am. J. Chin. Med. 45 (5) (2017) 965–985.
[115] A.K. Jha, et al., Perlecan domain I-conjugated, hyaluronic acid-based hydrogel particles for enhanced chondrogenic differentiation via BMP-2 release, Biomaterials 30 (36) (2009) 6964–6975.
[116] T. Nagai, et al., Bevacizumab, an anti-vascular endothelial growth factor antibody, inhibits osteoarthritis, Arthritis Res. Ther. 16 (5) (2014) 427.
[117] S. Lee, J.G. Nemeño, J.I. Lee, Repositioning bevacizumab: a promising therapeutic strategy for cartilage regeneration, Tissue Eng. B Rev. 22 (5) (2016) 341–357.
[118] M.M. Smith, A.J. Hayes, J. Melrose, Pentosan polysulphate (PPS), a semi-synthetic heparinoid DMOAD with roles in intervertebral disc repair biology emulating the stem cell instructive and tissue reparative properties of heparan sulphate, Stem Cells Dev. 31 (15–16) (2022) 406–430.
[119] M. Zhang, et al., Advanced application of stimuli-responsive drug delivery system for inflammatory arthritis treatment, Mater. Today Bio 14 (2022), 100223.
[120] M.C. Hochberg, et al., Effect of intra-articular sprifermin vs placebo on femorotibial joint cartilage thickness in patients with osteoarthritis: the FORWARD randomized clinical trial, JAMA 322 (14) (2019) 1360–1370.
[121] F. Eckstein, et al., Long-term structural and symptomatic effects of intra-articular sprifermin in patients with knee osteoarthritis: 5-year results from the FORWARD study, Ann. Rheum. Dis. 80 (8) (2021) 1062–1069.
[122] F. Eckstein, et al., Intra-articular sprifermin reduces cartilage loss in addition to increasing cartilage gain independent of location in the femorotibial joint: post-hoc analysis of a randomised, placebo-controlled phase II clinical trial, Ann. Rheum. Dis. 79 (4) (2020) 525–528.
[123] K.G. Auw Yang, et al., Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial, Osteoarthr. Cartil. 16 (4) (2008) 498–505.
[124] H.J. Kerkhof, et al., Large-scale meta-analysis of interleukin-1 beta and interleukin-1 receptor antagonist polymorphisms on risk of radiographic hip and knee osteoarthritis and severity of knee osteoarthritis, Osteoarthr. Cartil. 19 (3) (2011) 265–271.
[125] T.J. Schnitzer, et al., Effect of tanezumab on joint pain, physical function, and patient global assessment of osteoarthritis among patients with osteoarthritis of the hip or knee: a randomized clinical trial, JAMA 322 (1) (2019) 37–48.
[126] C.J. Malemud, Inhibition of MMPs and ADAM/ADAMTS, Biochem. Pharmacol. 165 (2019) 33–40.
[127] W. Zhang, et al., Emerging trend in the pharmacotherapy of osteoarthritis, Front. Endocrinol. 10 (2019) 431.
[128] A.J. Hayes, J. Melrose, Glycosaminoglycan and proteoglycan biotherapeutics in articular cartilage protection and repair strategies: novel approaches to visco-supplementation in orthobiologics, Adv. Ther. 2 (8) (2019), 1900034.
[129] X. Zhu, et al., Effectiveness and safety of glucosamine and chondroitin for the treatment of osteoarthritis: a meta-analysis of randomized controlled trials, J. Orthop. Surg. Res. 13 (1) (2018) 170.
[130] M.C. Hochberg, et al., Combined chondroitin sulfate and glucosamine for painful knee osteoarthritis: a multicentre, randomised, double-blind, non-inferiority trial versus celecoxib, Ann. Rheum. Dis. 75 (1) (2016) 37–44.

[131] N.K. Arden, et al., Non-surgical management of knee osteoarthritis: comparison of ESCEO and OARSI 2019 guidelines, Nat. Rev. Rheumatol. 17 (1) (2021) 59–66.
[132] P.M. Burt, et al., Ablation of low-molecular-weight FGF2 isoform accelerates murine osteoarthritis while loss of high-molecular-weight FGF2 isoforms offers protection, J. Cell. Physiol. 234 (4) (2019) 4418–4431.
[133] P. Meo Burt, L. Xiao, M.M. Hurley, FGF23 regulates Wnt/β-catenin signaling-mediated osteoarthritis in mice overexpressing high-molecular-weight FGF2, Endocrinology 159 (6) (2018) 2386–2396.