Perlecan: Roles in osteoarthritis and potential treating target

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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
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.

![Schematic illustration of the multidomain structure of human perlecan.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Interacting partner(s)</th>
<th>Potential functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>VEGF, PRELP, FGF, BMP-2, PDGF, HGF, SHH, WARP, Ang-3, LN, COL IV, V, VI, XI, II, III, IV, IL-8, IL-2, NID-1, FN, FBN1, activin A, TSP1</td>
<td>Angiogenesis; Cell adhesion and motility; Chondrogenesis; GF delivery and activation; ECM anchoring, assembly and stabilization</td>
<td>[13,19–28]</td>
</tr>
<tr>
<td>II</td>
<td>LDL, CTGF, Hes24, FBN1, Wnt/calcium binding and mediator Wnt/calcium signaling; BM anchoring; biogenesis of microfibrils</td>
<td>[13,19,29,30]</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>FGF7, FGF18, WARP</td>
<td>Cartilage structure assembly; ECM assembly/stabilization</td>
<td>[19,31]</td>
</tr>
<tr>
<td>IV</td>
<td>Heparin/sulfatide binding, FGF18, FN, NID1/2, PDGF, COL IV, FBLN-2</td>
<td>Cartilage development; BM stabilization</td>
<td>[13,19,24,32–34]</td>
</tr>
<tr>
<td>V</td>
<td>NID1, FGF-7, FBLN2, α-DG, PGRN, β1-integrin, COL VI, α2II1-integrin, TGF, VEGF, ECM-1, AchE</td>
<td>Angiogenesis; autophagy regulation; BM assembly/stabilization; cell proliferation</td>
<td>[13,19,20,28,35,36]</td>
</tr>
</tbody>
</table>

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.
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 (PlnDII)

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 HS binding site in neural cell adhesion molecules. HS binding is restricted to IG5 that low-affinity binding sites for FGF-18 are present in PlnDIII [31].

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 Pln with PRELP, nidogen-1, FGF-7 [35], fibulin-2 [28], COLIV, 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 H6ST1 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

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**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, downstream of which is not RUNX2 and ELK1.
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-1α–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.

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.
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 mechanical 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 cartilage degeneration [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, 5 A).

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 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.

![Diagram](image_url)

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.
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 hypertrophy [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 transcommunication, 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 ERBB 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 PlnI [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 fibrosis [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 osteocyte 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-1α significantly inhibited Fgfr1 expression to the same extent in both genotypes. In contrast, IL-1α decreased the expression of Fgfr3 to a significantly greater extent in WT mice than in Hspg2-2/2 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 osteocyte 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 FGFR3 [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 Adams-5, inflammation through the adjustment of IL-1β and subchondral bone formation through TGF-β1 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 ESCOE, 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 mouse 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 FGRF1 expression, whereas HMWKO cartilage exhibited increased levels of FGRF3 [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 hypertrophy, 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
SHI 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.

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