HHS Public Access

Author manuscript

Acta Biomater. Author manuscript; available in PMC 2022 November 01.

Published in final edited form as:

Acta Biomater. 2021 November ; 135: 13–26. doi:10.1016/j.actbio.2021.08.032.

Impact of perlecan, a core component of basement membrane, on regeneration of cartilaginous tissues

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Abstract

As an indispensable component of the extracellular matrix, perlecan (Pln) plays an essential role in cartilaginous tissue function. Although there exist studies suggesting that Pln expressed by cartilaginous tissues is critical for chondrogenesis, few papers have discussed the potential impact Pln may have on cartilage regeneration. In this review, we delineate Pln structure, biomechanical properties, and interactive ligands—which together contribute to the effect Pln has on cartilaginous tissue development. We also review how the signaling pathways of Pln affect cartilage development and scrutinize the potential application of Pln to divisions of cartilage regeneration, spanning vascularization, stem cell differentiation, and biomaterial improvement. The aim of this review is to deepen our understanding of the spatial and temporal interactions that occur between Pln and cartilaginous tissue and ultimately apply Pln in scaffold design to improve cell-based cartilage engineering and regeneration.

Graphical Abstract

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CRediT authorship contribution statement

Gongming Gao: Collected the data; Performed data analysis; Wrote the paper; Approved the submission. **Song Chen:** Collected the data; Performed data analysis; Wrote the paper; Approved the submission. **Yixuan Amy Pei:** Collected the data; Performed data analysis; Wrote the paper; Approved the submission. **Ming Pei:** Conceived and designed the analysis; Performed data analysis; Wrote the paper; Approved the submission; Supervision; Funding acquisition.

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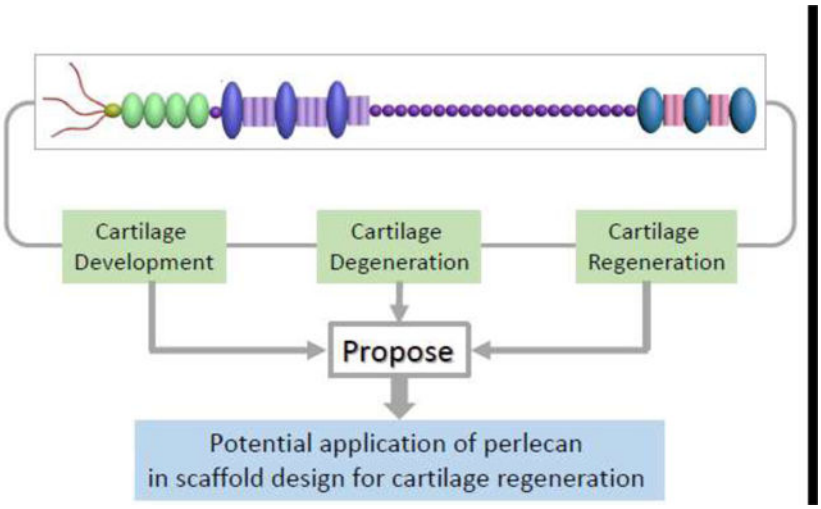
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.



Perlecan's potential application in cartilage regeneration.

Keywords

Perlecan; Cartilaginous tissues; Stem cell; Tissue regeneration; Pericellular matrix; Extracellular matrix

1. Introduction

Lacking blood vessels, nerves, and lymphatics, articular cartilage is a unique tissue with a limitation in intrinsic repair and healing. Articular cartilage, meniscus, and nucleus pulposus (NP) are cartilaginous tissues with unique properties but they share similar functionalities [1]. Extracellular matrix (ECM) secreted by chondrocytes is composed of type II collagen (COL II), glycosaminoglycan (GAG), elastin fibers, and laminin. Structurally, from the inside out, the ECM surrounding chondrocytes is divided into three layers: the pericellular matrix (PCM), the territorial matrix, and the interterritorial matrix [2].

Basement membrane (BM) is a ubiquitous structure of the ECM, which separates cells from and attaches them to the interstitial matrix [3] and exists in various tissues of the human body [4]. BM is an essential structure for maintaining various roles in tissue function, including but not limited to signal transduction, acting as a mechanical barrier, and tissue organization [5,6]. In chondrocytes, the distribution of BM components, including laminin, COL IV, nidogen, and perlecan (Pln), varies with age; initially widely distributed in the territorial and interterritorial matrix of mouse cartilage, BM components become primarily localized to the narrow PCM of the chondrocyte during maturation [7]. As such, during cartilage development, the PCM may constitute an integral ingredient of the dynamically modulated chondrocyte BM [7].

Perlecan (Pln), encoded by a heparan sulfate (HS) proteoglycan 2 (*HSPG2*) gene sited on the telomeric region of human chromosome 1 or of mouse chromosome 4, is one of the most abundant HSPGs in not only the BM but also many other tissues lacking typical BM

structure and blood vessels such as the cartilage, meniscus, and intervertebral disc (IVD) [8–10]. There exists strong evidence that Pln plays a critical role in brain, bone, heart, and cartilage development [11,12]. Mutations in the *HSPG2* gene result in two classes of skeletal disorders: Schwartz-Jampel syndrome, a mild disorder with increased risk of bone/cartilage loss, and Silverman-Handmaker type, a severe neonatal lethal dyssegmental dysplasia [13]. Pln deficiency is also a risk factor for osteoporosis [14].

Chondrocytes are enveloped by PCM, a narrow tissue region that, together with the enclosed chondrocytes, has been termed the “chondron”; the PCM, as a functional equivalent of BM, is distinguished from ECM in regard to its biochemistry, structure, and biomechanics [7]. In experimental settings where the HS chain of Pln is digested with heparinase III, no subsequent effect is observed on the elastic modulus of ECM, while the overall elastic modulus of both the general PCM and its interior regions increases [15]. This finding indicates that, besides COL VI, Pln can be considered as a defining factor exclusively localized to the PCM, which has also been found to have a significant effect on the structural integrity and biomechanical properties of PCM [15].

Given that *HSPG2* gene regulation, structure, and function in chondrogenesis have been well summarized [16–19], in this review, we will outline present knowledge of the biomechanical and biochemical roles Pln plays in cartilage and, specifically, the effects Pln has on the development of articular cartilage, meniscus, and spinal tissues. Considering that Pln was also found to be substituted with both HS and/or chondroitin sulfate (CS) [20,21], this review will further discuss the role of the Pln CS chain in cartilage regeneration and reconstruction. Finally, this review will elucidate Pln’s role within the signaling pathways involved in cartilaginous tissue development, which might provide insights in favor of cartilage regeneration and reconstruction.

2. Pln structures

Pln is a secreted HSPG that was first isolated in 1980 from Engelbreth–Holm–Swarm tumor cells [22]. The name Pln is derived from the pearls-on-a-string-like appearance as shown by rotary shadowing electron microscopy. Human Pln has a 467 kDa protein core and five well-defined domains (I through V) of which domain I is exclusive to the proteoglycan (PG), while domains II-V share homology with various biological components [23–25]. (Figure 1) (Table 1)

2.1. Domain-specific structures and interactions

Human Pln domain-I (PlnDI), the domain exclusive to Pln, contains three potential binding sites for HS and a sea urchin sperm protein, enterokinase, agrin (SEA) module [23,24,26,27]. PlnDI is capable of interacting with vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), bone morphogenetic factor-2 (BMP-2), platelet-derived growth factor (PDGF), hepatic growth factor, granulocyte-macrophage colony-stimulating factor, and angiopoietin-3 through its HS side chains [27]. Through these interactions, PlnDI activates various intracellular signaling pathways. Furthermore, PlnDI also interacts with components, such as sonic hedgehog, von Willebrand factor A domain related protein (WARP), proline and arginine rich leucine rich repeat protein, laminin, COL

IV, V, VI, and Xl, nidogen-1, fibronectin, thrombospondin-1, fibrillin-1, interleukin 2 (IL-2), IL-8, and activin A to stabilize the ECM and BM [27].

Pln domain II (PlnDII) contains four copies of internal repeats highly homologous to the low-density lipoprotein (LDL)-binding region of the LDL receptor [23]. Following the LDL receptor-like domain, there exists a short segment between domains II and III that is homologous to the repeats of immunoglobulin (Ig) that are also found in domain IV [23]. This Ig-like repeat can modulate calcium binding and mediate Wnt/calcium signaling [27]. In addition, PlnDII also interacts with very low-density lipoprotein, connective tissue growth factor/hypertrophic chondrocyte-specific gene product 24 (CTGF/Hcs24), and fibrillin-1 [28–30].

Pln domain III (PlnDIII) resembles the short arm of laminin chains [25]. It contains four internal cysteine-rich repeat subdomains that are separated by three laminin-like globular domains [23–25]. PlnDIII interacts with FGF-7/18, FGF-binding protein, and WARP [26,31–33].

Pln domain IV (PlnDIV) is the largest domain. Human PlnDIV contains 21 consecutive Ig-like repeats similar to those found in the neural cell adhesion molecule, whereas mouse PlnDIV only contains 14 consecutive Ig-like repeats [23,34,35], which decreases the core protein to 400 kDa [36]. This finding suggests that interspecies differences exist in the number of consecutive Ig-like subdomains. Additionally, the 14th Ig-like repeat of human Pln contains a potential GAG chain attachment sequence [24]. PlnDIV plays a crucial role not only in cartilage development through triggering chondrocyte condensation [37] but also in BM stabilization through interactions with fibronectin, nidogen-1/2, COL IV, fibulin-2, and PDGF [27].

Pln domain V (PlnDV) contains three laminin-like globular domains with two epidermal growth factors (EGF)-like domains among them; like PlnDIII, the three globular domains are similar to A chains of laminin [24,25]. Besides interacting with nidogen-1, FGF-7, fibulin-2, α-dystroglycan, progranulin, β1-integrin, ECM-1, acetylcholinesterase, and COL VI [27], PlnDV itself independently inhibits endothelial cell motility, tube formation, and blood vessel growth in angiogenesis assays [38].

The different domains each play a respective role in chondrogenesis. However, PlnDI and its binding GAG chains are often considered the most important as they provide the initial signal to activate chondrogenesis [39]. C3H10T1/2 cells, the mesenchymal fibroblast cell line, are able to enter chondrogenic differentiation when plated on the ECM protein Pln; however, these cellular Pln-stimulated nodules fail to express the chondrogenic maturation and subsequent terminal differentiation markers unless they are treated with BMP-2 [40]. This regulatory effect can be explained through two hypothesized mechanisms: (1) GAG chains could bind directly to cell surface receptors and stimulate cartilage differentiation through activating intracellular signaling pathways [39,41] and (2) GAG-bearing PGs could be able to serve as cell adhesion inhibitors that prevent cells from forming focal adhesions [39,42].

2.2. Tissue source-dependent structural variation

An interesting study comparing the effects of ECM from different cell sources on cartilage regeneration concludes that ECM differs in its function depending on the cell sources used [43]. Similarly, Pln from different cell sources is speculated to play distinctive roles in cartilage regeneration. Specialized cell-derived Pln has distinctive HS chain substructures, which may lead to differences in its ability to bind to growth factors [44]. Specifically, Pln derived from smooth muscle cells, IVD, and chondrocytes has both HS and CS chains, whereas Pln from endothelial cells contains exclusively HS chains [45,46]. Similarly, Pln from smooth muscle cells binds both FGF-1 and FGF-2 through its HS chains and promotes FGF-2 signaling but not FGF-1 signaling, while Pln from endothelial cells binds both FGF-1 and FGF-2 and promotes the signaling of both [45]. During BaF-32 cell (an IL-3 dependent and HSPG deficient myeloid B cell line [47]) proliferation, chondrocyte-derived Pln binds both FGF-2 and FGF-18 and forms HS chain-dependent ternary complexes with FGF-18 and FGF receptor 3 (FGFR3) [48]. Pln from endothelial cells can support BaF-32 cell proliferation with both FGF-1 and FGF-9, while Pln from chondrocytes is only responsive with FGF-9 [49]. Based on this differential bioactivity, it would be worthwhile to investigate which cell source would derive the most suitable version of Pln for use in cartilage regeneration, as there is no relevant research on the topic at present. The efficacy of adult stem cells in lineage-specific differentiation is greatly affected by the type of resident tissue from which they are isolated [50,51], which may expand our knowledge base.

3. Pln expression during development of cartilaginous tissues

As an essential component in these cartilaginous tissues [1], Pln plays a crucial role in developmental regulation.

3.1. Pln expression in the development of cartilage

When examining developing mouse embryos, approximately 40% of Pln-null mice at embryonic day 10.5 (E10.5) die as a result of defective cephalic development and about 60% of Pln-null mice die just after birth due to respiratory failure [52]. Only 6% of Pln-null mice develop both chondrodysplasia and exencephaly [52], which resembles Schwartz-Jampel syndrome [53]. However, loss of Pln does not affect early embryonic development, as results from E5.5 and E9.5 show that lack of Pln does not decrease the mouse embryo implantation rate [54]. Immunolocalization of Pln is first detected in angiogenic tissue at E10.5, earlier than in cartilaginous tissues; then from E11 to E13, Pln is abundantly expressed in developing cartilage undergoing endochondral ossification [55]. Genetic analysis indicates that, in normal limb bud cartilage, *HSPG2* mRNA is first detected at E13.0 and shows weak expression in hypertrophic chondrocytes at E14.0; in condylar cartilage, *HSPG2* mRNA is first detected in newly formed cartilage at E15.0 and declines in expression in the hypertrophic chondrocyte region [56]. Meanwhile, Pln immunoreactivity is evident throughout the tibial cartilage, indicating that these cells which develop into mature chondrocytes actively synthesize Pln, while hypertrophic chondrocytes downregulate Pln synthesis [56]. Loss of Pln reduces proliferative activity in E14.5 cartilage and aggrecan quantity in E16.5 cartilage, but has little effect on COL II [52]. Additional studies looking at human specimens have found that PlnDIII and V are expressed in cartilage anlagen and

zones of chondral ossification beginning at gestational week (GW) 8 [57]. After GW 12, Pln becomes strongly localized to developing cartilaginous tissues in humans [49].

As Pln supports cartilage maturation [55] and chondrocyte differentiation [58], Pln expression persists from postnatal, to juvenile, adolescent, and adult cartilage in human samples [49]. During this time, Pln may be present in forms possessing HS chains, CS chains, or both. During chondrocyte differentiation, Pln deposition is increased and the sulfation pattern of its CS chains changes depending on the different cell zones of the growth plate [59]. When Pln distribution is measured in aging cartilage—ranging from newborn to eight-year-old sheep—a significant age-dependent decline in Pln levels is observed in hyaline and growth plate cartilage [60].

3.2. Pln expression in the development of meniscus

As seen in articular cartilage, Pln is also expressed during the development of the meniscus, a wedge-shaped fibrous cartilage located in the knee joint. While immature meniscus is full of blood vessels and rich in blood supply, later in development, this blood supply gradually decreases [61]. In adulthood, the blood supply of the meniscus only exists in the peripheral 10%−30% [1]. Pln distribution in the meniscus may be partly related to its special blood supply structure, as Pln is a core component of vascular BM [47]. In a study examining the meniscus of merino sheep (from newborn to 19-month-old), Pln is found expressed in the inner third of the meniscus, co-localized with COL II and aggrecan [62]. A similar study based on the ovine lateral meniscus of merino sheep demonstrates that Pln exists in the inner and middle meniscal zones of 7- to 19-month-old samples but decreases in 7- to 10-year-old samples [63]. Interestingly, atomic force microscope (AFM) shows that micromechanical properties of the meniscus Pln-labeled PCM and ECM display regional variations; the elastic modulus of the Pln-labeled PCM in the outer region was significantly higher than the inner region, and ECM moduli were constantly higher than region-matched PCM sites in both the outer and inner regions [9].

3.3. Pln expression in the development of IVD

As a component of PCM, Pln is found in various embryonic and adult tissues, including the spinal tissues of animals and humans [52,55,58,64]. In developing mouse embryos, the initial accumulation of Pln can be detected in the IVD and basal lamina of surrounding tissues at E13.5, and high levels of Pln begin to accumulate in the cartilage primordium of these regions at E15.5 [58]. Similar research also demonstrates that Pln expression in the cartilage primordium of the vertebral bodies begins at E10.5 and becomes prominent by E13; by E14, Pln deposition is rich in the developing NP [55]. Immunolocalization in 12- to 14-week-old human fetal tissues further confirms that Pln is mainly distributed in NP and internal annulus fibrosus (AF) but shows weak expression in the tissues around the developing IVD and exhibits no expression in the notochordal tissues [64]. Interestingly, Hayes and Melrose recently detected Pln expression in the cytoplasm of both the NP and AF of the sheep IVD and proposed that Pln might be trafficked by transport vesicles connecting the cell nuclear and extracellular environment, indicating a novel transcriptionally regulatory site for the multifunctional roles Pln plays in the IVD [65,66]. In *HSPG2*$^{-/-}$ mice, cartilage and vertebral bodies show fibrous invasion from the perichondrium resulting in disruption

of the growth plate, suggesting that Pln plays a critical role in maintaining the integrity of the matrix structure [52]. Pln in human fetal spine is substituted with HS chains and the 7D4 CS sulfation motif; however, as the tissue matures, this epitope decreases in frequency and becomes virtually undetectable [67,68]. 4C3, 7D4, and 3B3[–] positive progenitor cell populations play important roles not only in IVD development but also in hematopoiesis, skin morphogenesis, and chondrogenesis [20].

Interactions between Pln and various ECM components contribute to matrix stabilization, cell-cell/ECM ligation and IVD cell involvement in the mechanical sensory processes that regulate tissue homeostasis and remodeling [69,70]. For example, elastin, an important ECM component located within translamellar cross bridges and between adjacent annular layers, plays a functional role in developing spinal tissues [71]. A series of related studies have discovered that elastic fiber-associated proteins fibrillin-1 and latent transforming growth factor beta (TGF-β) binding protein-2 (LTBP-2) are found in the annular lamellae in human fetal IVD and are associated with Pln [71]. Some literature shows that the Pln HS chains may contribute to fibrillin-1 assembly and distribution in the IVD [69] and LTBP-2 also appears to be co-localized with Pln in the outer layer of AF [71]. Notably, in the posterior layer of AF, Pln, and LTBP-2 are strongly co-deposited in the PCM of annular fibrochondrocytes [72]. In addition, Pln and COL VI, co-deposited in the PCM of IVD cells, are able to connect cells and their surrounding matrix into a network structure [73]. Furthermore, Pln can be helpful for chondrogenic differentiation of the developing IVD cells through the combination with FGF-18 and FGFR3 [68]. These results demonstrate that Pln is not only a marker of chondrogenesis [49], but also plays a crucial role in regulating early spinal tissue development.

4. Pln mediated biomechanical and biochemical impact on chondrogenesis

Consisting of five domains, each with its own structure and corresponding functions, Pln serves a multitude of complex biomechanical and biochemical roles in cellular maintenance and signaling pathways. Many domains contain homologous features with other biological components (i.e., laminin chain short arm, LDL receptor), as it is this structural adaptability that facilitates Pln's ability to bind and interact with a myriad of ligands in the PCM and ECM to activate intracellular signaling pathways as well as help in stabilizing the ECM and BM.

4.1. Biomechanical role of Pln in cartilage

Because mechanical signals are integral to cartilage development, pathophysiology, and regeneration [74], various cellular components have been studied to investigate their roles in creating these mechanical signals. As previously reported, the mechanical distribution in articular cartilage is heterogeneous and anisotropic at the microscopic level [75,76]. Stiffness mapping depicted with AFM indicates that the ratio of the modulus between PCM and ECM in the articular cartilage of different species—including human, porcine, and murine—is usually fixed; however, the modulus of ECM is significantly higher than that of PCM in said species [77]. A similar study examining porcine medial meniscus cartilage

has demonstrated that the elastic modulus is significantly elevated in the outer region of the Pln-labeled PCM when compared to the inner region [9]. Interestingly, most studies identify PCM of chondrocytes by COL VI labeling [78–80]; over time, Pln labeling has also been recognized as a reliable method to identify PCM [15]. Notably, regions in the PCM labelled by both COL VI and Pln were found to have lower elastic moduli than those labelled by Col IV alone, indicating that local Pln presence in the PCM lowers the elastic moduli [15]. However, in a murine model of the Schwartz-Jampel syndrome disorder, *HSPG2* knockdown results in abnormal PCM organization, altering these matrix properties and decreasing chondrocyte stiffness [81].

It has been shown that PCM may act as a regulator in mechanical-biological signaling transduction in chondrocytes, in which Pln plays an essential role [82–84]. *HSPG2* knockdown led to defective PCM formation and early onset of osteoarthritis (OA), whereas the concentration of ECM proteins, including many collagens, increased [85]. Physiologically related hydrostatic loading can significantly increase *HSPG2* gene expression in cartilage [86], and these changes vary based on the magnitude or duration of the applied loading. For example, in bovine cartilage, Pln expression is not altered after one cycle of physiologic, cyclic hydrostatic loading administered within one week, but does become increased after two and three cycles of loading [87]. Interestingly, Pln expression sensitivity to mechanical loading varies significantly depending on the age group from which the cartilage was derived. Under dynamic mechanical compression, a downregulation of Pln is observed in aged cartilage while no changes are observed in young cartilage [88]. This age-dependency is speculated to be regulated through Smad2/3P signaling and is associated with the mechanisms that lead to the development of OA [88]. Under mechanical loading, Pln is regulated through its interaction with FGF-2, which facilitates FGF-2's ability to initiate signal transmission quickly and allows Pln to directly participate in the signaling response during loading [84]. It is thought that, while in the resting state, FGF-2 is blocked on the cell surface and cannot bind to FGFR for signal transduction [84], but ECM deformation under mechanical loading allows the FGF-2-bound Pln to be presented to the cell surface receptor—thus activating downstream signaling pathways.

4.2. Biochemical role of Pln in chondrogenesis

Pln function is regulated by the interactions of the Pln core protein and GAG chains with different molecules in cell proliferation, adhesion, and nutrient metabolism [18,26,89,90]. Specifically, during chondrogenesis, specific growth factors and ligands (shown in Table 1) have been reported to be involved in Pln activity.

FGF—The human and mouse FGF gene families are comprised of 22 members [91,92]. Among these, FGF-2 and FGF-18 have been implicated in cartilage development [93].

FGF-2 has been reported to promote chondrocyte proliferation [94] and protect against development of OA by inhibiting a disintegrin and metallopeptidase with thrombospondin type 1 motif 5 (ADAMTS-5) formation [95]. FGF-2 participates in chondrocyte regulation by binding to FGFR1 and FGFR3—both of which play unique roles in the FGF-2 signaling pathways. The FGF-2/FGFR1 signaling pathway promotes deleterious activities such as

matrix metalloproteinase (MMP) activation and inhibition of ECM synthesis, while the FGF-2/FGFR3 signaling pathway increases cell proliferation and ECM production [46,93]. Pln regulates these pathways by interacting with FGFs/FGFRs to form a complex that later contributes to signal transduction [96]. Specifically, the FGF-2/Pln complex acts as a mechanotransducer [84]. As a low affinity receptor for FGF-2, Pln from developing growth plate chondrocytes is able to sequester FGF-2 away from the high affinity receptors on chondrocytes [97]. Immunohistochemical results show that Pln and FGF-2 are bound in all regions of cartilage, including weight-bearing and non-weight-bearing areas as well as the superficial zone and the mid zone of human and porcine cartilage [84]. Pln that is localized to the PCM of chondrocytes in developing growth plate cartilage rudiments contains HS chains and CS chains, each of which plays a distinctive role in binding FGF-2. While FGF-2 is able to freely bind to the HS chain of Pln, it is only able to be delivered to the FGFRs (FGFR1 and FGFR3) when the CS chain of Pln is removed [98].

As a regulator of chondrocyte proliferation, hypertrophy, and vascularization in the growth plate, FGF-18 plays different regulatory roles by binding to various FGFRs [99]. During long bone development, FGF-18 affects chondrogenesis through FGFR3 based signaling and osteogenesis through FGFR1 and/or FGFR2 based signaling [100,101]. In a cell culture system containing fetal rudiment chondrocytes, exogenous FGF-18 is able to enhance Pln expression in cartilaginous matrices [102]. PlnDIII is involved in binding FGF-18, and by doing so, Pln can change the mitogenic effect FGF-18 has on chondrocytes in the growth plate [103]. It has also been found that the HS chains of Pln modulate complex formation between FGF-18, FGFR3, and Pln; without FGF-18 participation, FGFR3 alone is unable to directly bind to the HS chains of Pln [48].

Other FGFs, such as FGF-9, are also capable of contributing to chondrogenesis. One instance can be seen with the sequential addition of FGF-2, FGF-9, and FGF-18, which helps to enhance the cartilage differentiation capabilities of human mesenchymal stem cells (MSCs) [104]. Interestingly, chondrocyte Pln bound FGF-1 and -9 appear to be less versatile than endothelial cell Pln. When examining BaF-32 cells transfected with FGFR3c, endothelial cell-derived Pln supports cell proliferation equally well with FGF-1 and -9, while chondrocyte-derived Pln only supports BaF-32 cell proliferation through the FGF-9/FGFR3c complex [49].

BMP-2—BMP-2 can effectively enhance MSC recruitment to cartilage condensation and initiate a BMP-dependent signaling pathway to induce chondrogenesis of mesenchymal progenitor cells [105]. As such, exposure to BMP-2 has been well documented to be important for articular cartilage development, maintenance, improved MSC chondrogenesis, and normal joint formation [106,107].

The interactions between BMP-2 and Pln have been shown to contribute to cartilage development. For example, C3H10T1/2 cells enter chondrogenic differentiation when plated on intact Pln or independent PlnDI; however, these Pln-stimulated cellular nodules fail to express chondrogenic maturation and subsequent terminal differentiation markers unless they undergo treatment with BMP-2 [40,108]. Thus, it is possible that BMP-2 treatment enhances the extracellular-signal-regulated kinase 1/2 (ERK1/2) activity involved

in chondrogenesis of C3H102T1/2 cells [109]; alternatively, HS chains of Pln can improve the biological activity of BMP-2 [110] and be more conducive to chondrogenesis than adipogenesis [108]. BMP-2 binding to PlnDI HS chains is necessary for coordinated chondrogenic differentiation [111]. In addition, the close combination of BMP-2 and PlnDI also improves osteogenic ability [112]. These functions are thought to stem from PlnDI's ability to act as a repository for BMP-2 storage, thus allowing for its controlled release, protecting it from degradation, and enhancing its biological activity [113,114].

CTGF—As a multifunctional secretory ECM protein with four domains, CTGF is highly expressed on hypertrophic chondrocytes in growing cartilage [115]. A myriad of studies have suggested that CTGF may play an integral role in many biological processes, affecting proliferation, angiogenesis, migration, differentiation, cell adhesion, wound healing, and cell-specific ECM protein synthesis [116–119]. Notably, despite being a hypertrophic chondrocyte gene product, CTGF itself does not stimulate articular chondrocyte hypertrophy [120]. In 2003, Nishida et al. found that CTGF co-localizes with Pln from chondrosarcoma derived HCS2/8 cells and that recombinant CTGF is able to dramatically promote *HSPG2* gene expression *in vitro* [28]. As CTGF is produced by hypertrophic chondrocytes in the hypertrophic region and Pln is also predominantly localized to the pre-hypertrophic region, it is possible that the role that CTGF plays in chondrocyte proliferation and differentiation may occur through Pln [28]. Outside of its interactions with Pln, CTGF is also capable of regulating chondrocyte proliferation and differentiation *via* BMP and mitogen-activated protein kinase (MAPK) signaling pathways [121].

WARP—Encoded by the VWA1 gene, WARP is a member of the von Willebrand factor A domain protein family in the ECM [122]. Though previous research claims that WARP is primarily expressed in articular cartilage and the IVD [31], immunohistochemical studies have shown that the distribution of WARP varies with cartilage development. During mouse embryonic development, WARP distribution in articular cartilage is restricted to the chondrogenous layers at embryonic day E15.5 and the PCM in the sixth week [31]. In the spine, WARP distribution is restricted to the articular surfaces of the vertebrae and PCM in the AF at E18.5 [31]. Besides co-localization with Pln by binding to domain III-2 core protein and domain I HS chains in cartilage [31], WARP also co-localizes with COL VI in the PCM of the superficial zone of articular chondrocytes [123]. This finding suggests that WARP may act as a bridge of communication between Pln and COL VI-labeled PCM in regulating human articular cartilage [123].

Outside of its role in cartilage, WARP is also expressed and co-stained with Pln in some non-cartilaginous tissues, including the vasculature of the central nervous system and muscle tissues, suggesting that WARP may help to maintain the function of a wide variety of Pln-containing tissues [124]. However, a study published in 2009 found that the absence of WARP does not cause abnormalities or affect BM formation in articular cartilage, IVD, or muscle tissue, rather it affects only the structure and function of the peripheral nervous system [125]. These studies indicate that WARP plays a specialized role in some BM structures, such as vasculature, muscle, and cartilage [126].

4.3. Roles of the Pln CS chain in chondrogenesis

Despite the fact that Pln is usually substituted with HS chains in vascular tissues [127], sometimes it is substituted with a hybrid form of HS and CS chains in chondrocytes and smooth muscle cells [128]. Infrequently, epithelial cell and chondrocyte-derived Pln is also a hybrid Pln and a unique PG containing HS, CS, and keratan sulfate (KS) chains [48,129]. CS sulfation motifs play important roles in the development of the human fetal hip, knee, and elbow joints and related connective tissues [20,130,131]. The fetal IVD progenitor cells also contain Pln changeably substituted with native 7D4 CS sulfation motif [67]. By decorating cell surface PGs on activated stem/progenitor cells, the CS sulfation motifs 4C3, 7D4, and 3B3[–] can be used to identify these cells in transitional areas of tissue development and in repair tissues [20,132]. Through binding, sequestration, or presentation of bioactive signaling molecules, such as FGF, CS sulfation motifs participate in modulating the signaling gradients in charge of the cellular behaviors, including but not limited to differentiation, proliferation, and matrix turnover, which shape the zonal tissue architecture existing in mature articular cartilage [130].

5. Signaling pathways of Pln and effect on cartilage development

Pln's unique localization and binding interactions induce many intricate signaling pathways with downstream targets that significantly impact chondrocyte gene expression and the surrounding mechanotransduction. (Figure 2) As such, Pln is seen to play an integral role in regulating cartilage behavior throughout its genesis and development, including but not limited to: prechondrogenic cell behavior, catabolic and anabolic regulation of cartilage homeostasis, chondrocyte proliferation and differentiation, and cartilage tissue vascularization.

5.1. FGF signaling and mechanotransduction role

Current studies have shown that Pln plays a key role in post-traumatic OA progression. The ablation of PlnDI HS could inhibit articular cartilage degradation, decrease synovial inflammation, and reduce osteophyte size [46]. In cartilage, Pln not only participates in FGF delivery to cell surface receptors, but also sequesters FGFs from the PCM, acting as a mechanotransducer during cartilage loading [15,84] or in response to cartilage injury [133,134].

Two members of the FGF family, FGF-2 and FGF-18, are important regulators of cartilage homeostasis. It has been demonstrated that chondrocyte-derived Pln HS regulates cartilage homeostasis by participating in tripartite complex formation with both FGF-18 and FGFR3 as well as FGF-2 and FGFR1 or FGFR3 [48]. FGF-2 selectively activates FGFR1 to upregulate the production of matrix-degrading enzymes and inhibit the synthesis of ECM and PGs [135,136]. Thus, in adult human articular chondrocytes, FGF-2 takes on a catabolic role [93]. The interactions between FGF-2 and FGFR1 independently activate Ras and protein kinase C (PKC), both of which are integrated by Raf-MEK1/2-ERK1/2 and thus concertedly drive signaling [93,137]. ERK1/2 activates at least two key downstream transcription factors [i.e., Ets-like protein-1 (*ELK1*) and runt-related transcription factor 2 (*RUNX2*)] that upregulate the expression of a series of matrix-degrading enzymes [137]. On

the other hand, FGF-18 binds to FGFR3 and then activates downstream MAPK and protein kinase B (Akt) signal pathways [93,138]. The MAPK signal pathway can inhibit *ELK1* and *RUNX2* expression which decreases ECM degradation, while the Akt pathway promotes ECM formation and chondrocyte differentiation [93]. Hence, the downstream effects of FGF-18 ultimately promote anabolic activity in human articular chondrocytes [93].

FGF-2 has been shown to influence Pln-regulated mechanotransduction in cartilage *via* the ERK signaling pathway [84,134,139]. The elastic moduli of the PCM is lower in Pln-rich regions than in Pln-absent regions. This lower elastic modulus is thought to arise from the Pln HS chain interactions, as the enzymatic removal of HS chains drastically enhances PCM's microscale elastic properties while exhibiting little influence on ECM properties [15]. In unloaded cartilage, the Pln HS chain bound FGF-2 is sequestered from the cell surface, but upon matrix deformation, HS-bound FGF-2 is presented to its corresponding cell surface receptor, thus activating the ERK signaling pathway [84]. Besides this mechanism, other pathways have also been documented to contribute to the chondrocyte response to loading. For instance, in 2007, loading-induced protein tyrosine phosphorylation was found to be FGF dependent [84]; briefly, the authors found that FGF-2 and perlecan co-located in the PCM of articular cartilage; ERK activation upon loading was dependent on pericellular FGF-2 rather than release of intracellular growth factors. They also found that FGFR inhibitor could markedly suppress the increase in the level of protein tyrosine phosphorylation upon loading. These findings indicate that FGF-2/Pln is involved in signal transduction under stress stimulation.

5.2. BMP signaling

BMP-2 is essential to bone and cartilage formation [140]. The interactions between BMP-2 and Pln have been shown to contribute to cartilage development [111,113,141] and improve osteogenic ability [112]. BMP-mediated developmental processes are known to depend on the basic N-terminal domains of dimeric BMP-2 that serve as heparin-binding sites; although these sites are not integral to receptor co-activation, they help to regulate its biological activity [142]. Jiao et al. demonstrated that HSPG modulates the osteogenic activity of BMP-2 by sequestering BMP-2 at the cell surface so that it is unable to bind to the BMP receptor [143]. PlnDI can thus serve as a depot for BMP-2 storage, allowing it to be released in a controlled manner, protecting it from degradation, and enhancing its biological activity [113,114].

Both the canonical Smad-dependent pathway—involving TGF-β/BMP ligands, receptors, and Smads—and the non-canonical Smad-independent pathway—involving p38 MAPK—have been identified in chondrocytes [144,145]. Most BMPs phosphorylate and activate R-Smads (Smad1/5/8) to complex with Co-Smad (Smad4); the Smad1/5/8-Smad4 complex subsequently translocates into the nucleus to regulate *RUNX2* and SRY (sex determining region Y)-box 9 (*SOX9*) target gene expression [146,147]. Interestingly, p38 MAPK is also involved in fine-tuning BMP's impact on chondrocytes and osteoblasts through TGF-β-activated kinase 1 (TAK1) [148]. In the postnatal chondrocyte, TAK1 deletion exhibits severe growth retardation and reduced SOX protein, PG, and COL II expression [149]. In contrast, in osteoblasts, TAK1 deletion leads to clavicular hypoplasia and postpones

fontanelle fusion, resulting in a phenotype alike to that seen in cleidocranial dysplasia—a disorder resulting from RUNX2 deficiency [150].

The roles that BMP signaling plays in chondrogenesis and osteogenesis are regulated by the delicate balance between ligands and antagonists—including Chordin, Chordin-like (CHL), Fellistatin, gremlin 1 (Grem1), and Noggin [144]—and these BMP antagonist expression patterns vary depending on joint location and species [106].

5.3. CTGF signaling

As a multifunctional secretory ECM protein, CTGF is highly expressed by hypertrophic chondrocytes in growth cartilage [115]. Not only does CTGF co-localize with Pln from HCS2/8 cells, but recombinant CTGF can also promote the *in vitro* expression of the *HSPG2* gene dramatically [29]. The LDL receptor is known to directly interact with CTGF [151,152]. Recently, it has been shown that PlnDII, which contains repeat sequences highly similar to those of the LDL receptor, can also bind CTGF in the PCM of articular cartilage [153]. As such, CTGF may be able to promote chondrocyte proliferation and differentiation through its interaction with Pln [29].

The interaction between CTGF and Pln triggers various intracellular signaling pathways. PKC acts as a central upstream kinase in chondrocyte proliferation, maturation, and terminal differentiation through its triggering of MEK, p38 MAPK, and PKB, respectively [121]. CTGF also activates the JNK pathway, which triggers chondrocyte proliferation and maturation [121]. In promoting chondrocyte terminal differentiation, PKB-mediated activation of phosphoinositide 3-kinase (PI3K) is also required [121,154,155].

5.4. FAK-Src signaling

PlnDIV prevents early cell attachment and promotes cell clustering by suppressing focal adhesion kinase/steroid receptor co-activator (FAK/Src) activity, with increased mRNA levels of chondrogenic markers *SOX9*, Cadherin 2 (*CDH2*), type II collagen (*COL2A1*), and aggrecan (*ACAN*) [37]. These markers are uniquely indicative of different milestones in chondrogenic differentiation. For instance, CDH2 is a precartilage condensation marker and downstream target of *SOX9* [156]. The regulatory role that FAK-Src signaling plays in cell adhesion and spreading occurs *via* the loss of phospho-ERK1/2 [37]. Forkhead box protein M1 (FoxM1), a downstream target of ERK, is involved in cell cycle progression and is downregulated as a result of PlnDIV-induced clustering, along with increased expression of cyclin-dependent kinase inhibitor 1C (*CDKN1C*) and activating transcription factor 3 (*ATF3*) [37]. Overexpression of ATF3 in chondrocytes then results in downregulation of cyclin D1 and cyclin A expression [157]. As such, the FAK-Src pathway culminates with chondrocyte proliferation suppression [37]. These findings described the mechanism of how PlnDIV affects prechondrogenic cell condensation and prevents chondrocyte progression to fibrocartilage.

5.5. VEGF signaling

In the early stages of cartilage development, angiogenesis helps to provide nutrients to the immature cartilage. As a component of vascular BM, Pln contributes to the process of

angiogenesis [158] and wound healing [159]. Furthermore, studies have pointed out that VEGF is necessary in cartilage angiogenesis during cartilage development and chondrocyte differentiation during endochondral bone formation [160–162]. Thus, regulation of VEGF expression is important in maintaining the chondrocyte phenotype. Pln can play promoting and/or inhibiting roles during angiogenesis [163–165], and VEGFR1 and VEGFR2 are the primary receptors involved in angiogenic activities of VEGF [166]. Early studies have shown that full-length Pln and immobilized forms of PlnDI can adjust the VEGF-VEGFR2 signaling pathway to regulate developmental angiogenesis [167,168]. Muthusamy and colleagues later found that the soluble forms of recombinant PlnDI, unbound or bound to VEGF165, stimulate angiogenesis by direct interactions with: 1) neuropilin 1, 2) VEGFR2, and 3) neuropilin 1 and VEGFR2 co-receptors [164]. It was later proven that cartilage Pln is a necessary component in the activation of VEGFR signaling in endothelial cells that ultimately leads to vascular invasion into the cartilage [169]. As Pln is a component of the surface vessel network, which provides nutrients to the developing joint rudiment, Pln regulation is likely beneficial to the activation of angiogenesis in early cartilage development [47].

The C-terminal fragment of Pln (PlnDV), called Endorepellin, contains three laminin globular domains (LG1/2/3) and is responsible for facilitating the role Pln plays in inhibiting angiogenesis [163,165,170]. LG1/2 domains bind to VEGFR2, inhibiting its VEGF-mediated activation by blocking Tyr1175 phosphorylation. This inhibition blocks endothelial cell migration and *VEGF* gene transcription, both of which contribute to angiostasis [165]. The LG3 domain, on the other hand, directly binds to α2β1 integrin, which also contributes to anti-angiogenesis [171]. The mechanism involves the activation of Src homology-2 protein phosphatase-1 (SHP-1), which dephosphorylates Tyr1175 of VEGFR2 and induces cytoskeletal collapse [171]. Both VEGFR2 and α2β1 integrin are required for Endorepellin angiostatic activity and the internalization and degradation of the dual receptor is mediated by the caveosome pathway [163,165].

6. Potential applications and challenge

Pln has been shown to be not only responsible for much of the biomechanics that characterize its matrices, as well as displaying cell source-dependent functionality that affects its ability to facilitate matrix mechanics. This structural variability lends Pln great potential for use in biomaterial synthesis. To this end, we explore current literature to speculate about the potential involvement of Pln in cartilage regeneration following injury and potential applications of Pln in stem cell and biomaterial mediated cartilage engineering.

6.1. Pln may mediate cartilage regeneration following injury

Given its impact on angiogenesis, wound healing, and maintaining cartilage function, Pln might be positively associated with cartilage regeneration. A study analyzing fresh bovine cartilage specimens found a significant negative correlation between age and *HSPG2* mRNA expression [87]. Interestingly, in human knee OA cartilage, Pln levels are significantly higher in areas close to cartilage defects [172]. Similarly, stimulation of equine cartilage with IL-1β has been proven to upregulate Pln expression [173]. Furthermore, a 2015 study

found that HSPG breakdown during inflammatory and mechanical injury in serious cases of OA leads to the disruption of the ELR+ CXC chemokine signaling pathway, contributing to loss of chondrocyte phenotypic stability. This process is due to HSPG's function in retaining C-X-C motif chemokine ligand 6 (CXCL6), a compound that has the capacity to attract inflammatory cells when released from the ECM and is linked to inflammatory arthritis [174]. Pln's chondroprotective role is thought to stem from Pln's ability to modulate FGF signaling [84,175].

Much evidence also points to the potential utilization of Pln as a regulator in stem cell chondrogenic differentiation. As mentioned above, GAG chains of Pln can interact with various ligands to regulate chondrogenesis. Exogenous HS has been proven to enhance TGF-β3-induced chondrogenic differentiation of human MSCs by triggering the Smad2/3 signaling pathway [176]. Furthermore, a series of studies conducted by Sadatsuki and colleagues concluded that Pln in the synovial niche is essential for chondrogenesis of synovial mesenchymal cells, but not for osteogenesis and cell proliferation [177]. This Pln-induced synovial mesenchymal cell chondrogenic differentiation primarily depends on the regulation of *SOX9* gene expression [178] as well as contributions from the Smad and MAPK signaling pathways [179].

Despite the promotion of developing and mature cartilage tissues, Pln also retains permanent cartilage by preventing further development, indicative of its role in tissue homeostasis. Shu and coworkers found that the use of heparanase in chondrocyte cultures increased cell proliferation and GAG production. Furthermore, they found that growth plate maturation, GAG deposition, and cell proliferation and maturation significantly enhanced in the Pln exon 3 null mouse model IVD, demonstrating the repressive control of the Pln HS chain [10]. The function of the Pln HS chain in tissue stabilization was also found in the PCM of AF cells and chondrocytes through interaction with COL XI [180] and in the small blood vessels of the synovium *via* interaction with elastin and fibrinlin-1 [181].

6.2. Pln potential applications in scaffold design

Ideal characteristics of a biomaterial that is to be used in cartilage regeneration necessitate the material's biocompatibility, bioactivity, biomimetics, biodegradability, and bioresponsiveness [182]. Given Pln's unique structure, localization, and distinctive biological activity displayed by each of the five different domains, some scholars [183] believe that it is an ideal candidate for modifying and enhancing the extracellular scaffold, directly linking the ECM to the cell surface and initiating cellular signals. Domain I, in particular, is recognized to have well-defined functionality in cartilage regeneration. Previous research shows that chitosan scaffolds containing PlnDI have the potential to induce wound healing [159]. PlnDI-COL II fibril modified polylactic acid scaffolds support chondrogenesis of both primary mouse embryonic fibroblasts and C3H10T1/2 cells *via* the binding and retention and release of BMP-2 [141]. Electrospun collagen fibers coated with PlnDI are able to bind to FGF-2 at rates that are ten times that of heparin-bovine serum albumin collagen fiber; this finding demonstrates a potential application of Pln-enhanced materials in constructing tissue engineering scaffolds [184]. Furthermore, combining a hyaluronic acid-based hydrogel with PlnDI has proven to make up an effective system for

BMP-2 delivery that is able to promote chondrogenic differentiation *in vitro* [111] and serve as an injectable therapeutic agent for OA treatment *in vivo* [113]. As indicated in Table 2, growing evidence suggests that Pln functionality is not limited to use in cartilaginous tissues, but rather it has broad application in enhancing biomaterials for cell-based therapies.

7. Conclusions and perspective

As a key component of stem cell niches, Pln is part of a highly complex microenvironment that promotes cartilage regeneration [131], which is supported by the presence of Pln in repaired human cartilage tissue through cell therapy and natural occurrence in a recent report [185]. In this review, we have compiled growing evidence that Pln in the PCM of cartilaginous tissues is involved in the regulation of biomechanical properties that characterize its various matrices and may thus have unique application in cartilaginous tissue regeneration. Although increasing evidence suggests that the PCM may serve as a mechanical-biological signal transducer in chondrocytes, the specific role that Pln plays in various mechanical-biological signaling pathways is still unclear. Based on the features mentioned above, Pln appears to have great potential for improving the cell culture environment. However, current efforts to introduce Pln to tissue engineering have been limited to the use of Pln-coating in cell culture environments—such applications prove to be insufficient given Pln's potential as a biomaterial. Given that cell-derived decellularized ECM—an excellent *in vitro* 3D culture scaffold model—can rejuvenate stem cell proliferation and chondrogenic differentiation [186–188], which has been used to define the impact of fibronectin on stem cells' proliferation and differentiation [189], it might provide an excellent *in vitro* model to delineate the role Pln plays in this matrix microenvironment.

Despite various efforts to illuminate Pln's role in chondrogenesis and cartilage regeneration, our understanding of Pln function remains limited. PlnDI is unique to Pln and many studies focus on this domain; however, the functions of other domains of Pln lack attention, which might be the focus of future investigation. In practical terms, the reproduction of specific HS sequence information on recombinant Pln domains is technically problematic especially since the specific HS sequences that drive repair are incompletely understood, despite that recombinant PlnDI and PlnDV forms containing GAG have been prepared/used successfully in a number of tissue repair strategies [111,113,184,190,191].

Besides cartilage regeneration, the role of Pln in cartilage degeneration also deserves attention. For instance, a 2013 study using a synovial *HSPG2*-deficient mouse model found synovium-localized Pln to be required in osteophyte formation in OA [192]. Furthermore, a 2015 study found that blocked endogenous Pln function improves differentiation of rat articular chondrocytes *in vitro*, suggesting that Pln may promote the dedifferentiation of chondrocytes; it should be noted that this result is only seen in environments favoring dedifferentiation (i.e., canonical Wnt signaling and low levels of BMP-2 and BMP-4) [193]. Because the degenerative contribution of Pln has only been observed in experiments testing a pre-existing degenerative microenvironment, it is likely that Pln only serves a passive role in cartilage degeneration. Hence, evidence that Pln actively triggers catabolic responses is still lacking and deserves further study.

Acknowledgements

We thank Suzanne Danley for editing the manuscript. This work was supported by Research Grants from the National Institutes of Health (1R01AR067747) to M.P. and Health Commission of Sichuan Province (18PJ008) and Science & Technology Department of Sichuan Province (2019YFS0267) to S.C.

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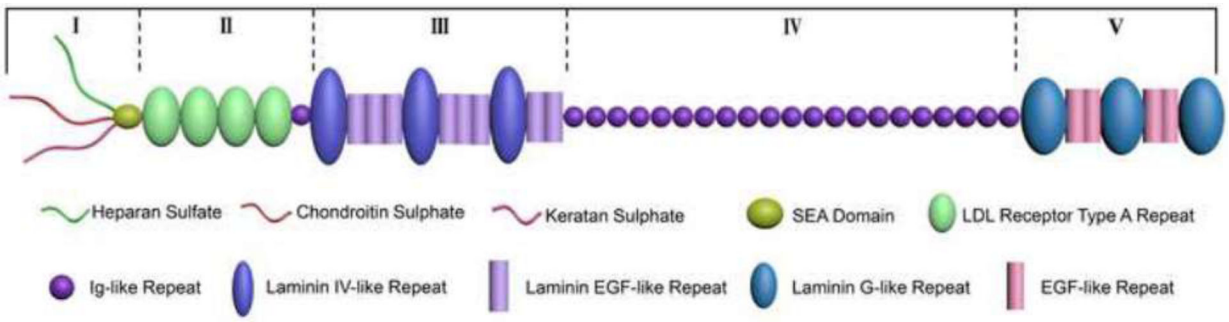

Figure 1.

Schematic illustration of the multidomain structure of human Pln. The core protein of Pln consists of five domains I–V [194]. Domain I is made up of a sperm, enterokinase and agrin (SEA) fold, preceded by three GAG attachment sites. Domain II contains four low-density lipoprotein (LDL) receptor motifs and one isolated immunoglobulin-like (Ig) fold. Domain III is made up of a combination of laminin epidermal growth factor (EGF) and laminin IV type A domains that are connected by disulfide linkages in the laminin EGF domains to form an inflexible rod-like structure. In humans, domain IV consists of 21 repeating Ig C2-type modules that are sequentially linked together. Domain V comprises four EGF motifs, three laminin G, and a fourth variable GAG attachment site.

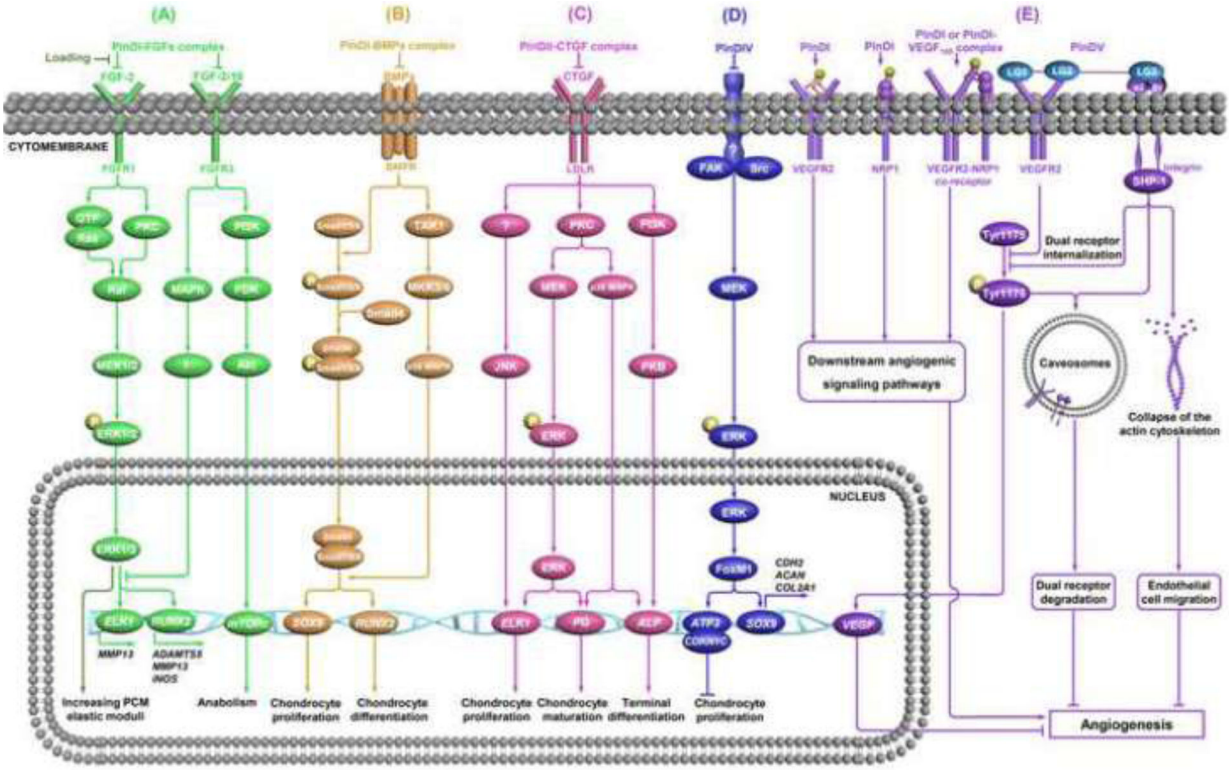

Figure 2.

Signaling pathways of Pln and effect on cartilage development. **(A) FGF signaling and mechanotransduction role.** The role Pln plays in regulating cartilage homeostasis is achieved by the formation of the PlnDI-FGF complex. This complex sequesters FGFs from receptors on the cell surface and inhibits the downstream signaling pathway [48,93,135–137]. FGF-2 also plays a mechanotransduction role in cartilage *via* influencing the ERK signaling pathway [15,84,134,139]. **(B) BMP signaling**. The roles that BMP signaling plays in chondrogenesis and osteogenesis are regulated by BMP-2 storage in the PlnDI-BMP complex and the delicate balance between ligands and antagonists [143–147,149]. **(C) CTGF signaling**. The interaction between CTGF and PlnDII triggers various intracellular signaling pathways and thus regulates the proliferation and maturation of chondrocytes [29,121,151,152,154,155]. **(D) FAK-Src signaling.** PlnDIV prevents early cell attachment and promotes cell clustering/condensation by suppressing the FAK/Src signaling pathway, but the receptor is unknown [37,156,157]. **(E) VEGF signaling**. In the early stage of cartilage development, PlnDI, unbound or bound to VEGF165, stimulates angiogenesis by direct interactions with neuropilin 1 (NRP1), VEGFR2, and NRP1/VEGFR2 co-receptors [164]. In the later stage of cartilage development, PlnDV inhibits angiogenesis *via* dual receptor VEGF2 and integrin. The endorepellin-induced dual receptor internalization and degradation by the caveosome-mediated pathway is also involved in angiostasis [163,165,171].

Table 1.

The protein domains of perlecan and their functions through interactive ligands.

Domain	Interacting partner(s)	Potential function	References
I (HS)	Activin A, Ang-3, BMP-2, FBN1, FGF-2/18, GM-CSF, HGF, VEGF, WARP, PRELP, LN, PDGF	Angiogenesis; Cell adhesion/motility; Chondrogenesis; GF delivery/activity; ECM anchoring/assembly/stabilization	[27,30,31,48,141,195–198]
II	CTGF, FBN1, LDL/VLDL, Wnt/Ca2+	BM anchoring and biogenesis of microfibrils	[27–30]
III	FGF-7/18, PDGF, WARP	Angiogenesis; Cartilage structure assembly; ECM assembly/stabilization; GF delivery	[31–33,199]
IV	COL IV, FBLN2, FN, Heparin, LN, NID1/2, PDGF	Angiogenesis; BM stabilization; GF reservoir	[27,200,201]
V	α2β1 Integrin, DG, ECM1, FBLN2, FGF-7, Heparin, NID1, PGRN, PRELP	Angiogenesis; BM assembly/stabilization; Cell adhesion and motility; Proliferation	[27,32,195,202,203]

Abbreviation: 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; GM-CSF: granulocyte-macrophage colony-stimulating 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; VEGF: vascular endothelial growth factor; VLDL: very low-density lipoprotein; WARP: von Willebrand factor A domain related protein

Table 2.

Positive effects of perlecan on biomaterials improvement.

Species	Cell/tissue location	Modified biomaterials	Result	References
Human	MG-63 cell	Coating electrospun collagen and gelatin fibers with PlnDI	Increasing FGF-2 binding	[184]
	BMSC/MG-63 cell	PlnDI-containing COL I scaffold	Promoting cell proliferation by enhancing FGF-2 activity	[204]
	C4-2B cell	Electrospun PCL-based scaffolds with PlnDIV	Facilitating cell proliferation, survival, and migration	[205]
	Coronary artery endothelial cell	Silk biomaterials functionalized with PlnDV	Supporting cell adhesion, spreading, and proliferation	[206]
Mouse	Cartilage	PlnDI immobilized to HA microgel	Repairing OA-like damage by controlled release of BMP-2	[113]
	C3H10T1/2 cell	PlnDI-conjugated, HA-based HGPs	Stimulating chondrogenic specific ECM production by binding and sustained release of BMP-2	[111]
		PlnDI and COL II coated PLA scaffold	Promoting cartilage-like tissue in PlnDI/COL II coated PLA scaffold	[141]
	BMSC	PlnDI-functionalized electrospun PCL scaffolds	Enhancing ALP activity by increasing PCL loading capacity of BMP-2	[207]
	RGC-5 cell	Biological glue made by mixing laminin, COL IV, entactin, and Pln	Improving retina adhesion rate	[208]
Rat	Skin	PlnDI-containing chitosan scaffolds	Promoting angiogenesis and dermal wound healing	[159]
	Bone	A recombinant PlnDI expressed from HEK 293 cells with HS/CS PG tightly binding recombinant human BMP-2	Promoting dose-enhancement of BMP-2 on a particulate TCP scaffold to generate up to 9 fold more bone volume in 6 or more weeks in a rat model than the control without PlnDI	[112]

Abbreviation: ALP: alkaline phosphatase; BMP-2: bone morphogenetic protein 2; BMSC: bone marrow stromal cell; C3H10T1/2: a murine mesenchymal stem cell line; C4-2B: a cancer cell line; CS: chondroitin sulfate; ECM: extracellular matrix; FGF-2: fibroblast growth factor 2; HA: hyaluronic acid; HGP: hydrogel particle; HS: heparan sulfate; MG63: an osteoblastic cell line; OA: osteoarthritis; PCL: poly (epsilon-caprolactone); PLA: polylactic acid; PG: proteoglycan; RGC-5: a retinal ganglion cell line; TCP: tricalcium phosphate