

Cartilage intermediate layer protein affects the progression of intervertebral disc degeneration by regulating the extracellular microenvironment (Review)

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Abstract. Intervertebral disc degeneration (IDD), which is caused by multiple factors, affects the health of individuals and contributes to low back pain. The pathology of IDD is complicated, and changes in the extracellular microenvironment play an important role in promoting the process of degeneration. Cartilage intermediate layer protein (CILP) is a matrix protein that resides in the middle of human articular cartilage and is involved in numerous diseases that affect cartilage. However, there is no detailed review of the relationship between CILP and degenerative disc disease. Growing evidence has revealed the presence of CILP in the extracellular microenvironment of intervertebral discs (IVDs) and has suggested that there is a gradual increase in CILP in degenerative discs. Specifically, CILP plays an important role in regulating the metabolism of the extracellular matrix (ECM), an important component of the extracellular microenvironment. CILP can combine with transforming growth factor- β or insulin-like growth factor-1 to regulate the ECM synthesis of IVDs and influence the balance of ECM metabolism, which leads to changes in the extracellular microenvironment to promote the process of IDD. It may be possible to show the correlation of CILP with IDD and to target CILP to interfere with IDD. For this purpose, in the present study, the current knowledge on CILP was summarized and a detailed description of CILP in discs was provided.

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1. Introduction

Low back pain (LBP) is one of the most prevalent musculoskeletal diseases worldwide. Approximately 70-85% of adults suffer from LBP in their lifetime, and a great number of them are disabled by it (1). The cost for treatment can reach billions of dollars, creating a huge burden for the families of patients and society (2). However, as a result of the complex pathology of LBP and the poor performance of current therapeutic measures, LBP still constitutes a major threat to the health of people.

There are numerous pathogenic factors leading to LBP, with intervertebral disc degeneration (IDD) being the most common target for diagnosis and intervention (3-5). As the largest avascular and aneural tissue in the human body, the normal intervertebral discs (IVDs) are made up of three morphologically distinct regions, the nucleus pulposus (NP), annulus fibrosus (AF) and cartilaginous endplates (CEPs) (6-9). IVDs function through dampening excessive mechanical stresses and maintaining the stability of the spine (10). It is important to provide thorough insight into the complicated pathophysiological process of IDD, in order to develop a strategy for the prevention and treatment of LBP. IDD is a multifactorial result characterized by an aberrant cell-mediated response that gradually causes structural failure (11). Aberrant cell-mediated responses to the changed microenvironment include an imbalanced extracellular matrix (ECM) metabolism, an upregulated

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proinflammatory phenotype and senescence (12-15). During this process, the upregulation of proinflammatory and procatabolic phenotypes by NP cells is the main contributor to the suppression of anabolism and promotes the catabolism of the ECM (16,17).

Cartilage intermediate layer protein (CILP) is a monomeric glycoprotein residing in the ECM that is mainly expressed in the intermediate zone between human IVDs and articular cartilage (18-20). Previous studies have also revealed the existence of a tendon ligament (21) and a synovial membrane (22). Notably, CILP expression in disc tissues has been revealed to be increased as degeneration and aging progress, contrary to the decreased levels of collagen II and aggrecans, which are the main components of discs (23). Furthermore, recent studies have revealed that CILP-overexpression in human NP cells can negatively regulate matrix synthesis (24). In the aberrantly expressed genes detected in IDD, CILP is among the few cartilage matrix proteins whose expression is upregulated in the early and late stages of cartilage diseases (19,25), and a genetic association has been revealed between the CILP gene and IDD, suggesting the importance of CILP beyond that of other structural genes (26). The relationship between CILP and IDD has received increasing attention in recent years. Herein, insight was first provided concerning the pathogenesis of IDD, and the genetic and molecular structure of CILP was described. Next, a detailed introduction of the function of CILP in IVDs was provided, and the genetic structure of the association between CILP and cartilage diseases was described. The regulatory mechanism of CILP was then summarized. Finally, the discussion focused on the future perspectives of CILP in biological therapies for IDD.

2. Intervertebral disc degeneration (IDD) pathogenesis

IDD is a multifactorial result caused by aging, infection, smoking, mechanical overloading, nutrient deficiency and genetic predisposition (27-31). Among the numerous factors that lead to IDD, the destruction of extracellular microenvironmental homeostasis is considered to be one of the most important factors. All the etiologic causes initiate the process of IDD, which is mediated and characterized by an enhanced proinflammatory phenotype (32-34). The increased inflammatory chemokine level, secreted from disc cells, infiltrates immune and AF cells, greatly destroying the homeostasis of the microenvironment around disc cells and directly affecting the metabolism of NP cells, which leads to a disruption of the balance between the anabolism and degradation of the ECM that directly accelerate the degradation of the ECM (35-38). As a result, the resident cells are exposed to excessive mechanical stress, which in turn further worsens the ECM metabolism of NP cells (24). In addition, the nociceptive nerve fibers and blood vessels from the dorsal root ganglion intrude into the herniated disc tissues to cause LBP (Fig. 1) (39,40). Notably, gene susceptibility has also been revealed to be involved in the initiation and progression of IDD; CILP is among the susceptible genes that are aberrantly expressed in IVDs (41,42). Moreover, CILP is restrictively expressed in few cartilage tissues, including articular cartilage and disc tissues (18,26), which suggests the importance of CILP beyond other susceptibility genes, elucidates the

function of CILP and contributes to a better understanding of the pathogenesis of IDD.

3. Structure and synthesis of CILP

CILP was first identified and isolated by Lorenzo *et al* in 1998; this protein was named for its deposition in the interterritorial matrix without a presence in the superficial or deepest regions of the articular cartilage (18). CILP is synthesized by cartilage chondrocytes and is a polypeptide of 1,184 amino acids with a molecular mass of 132.5 kDa. Apart from a putative signal peptide of 21 amino acids, the protein is comprised of 2 distinct polypeptides (20). The N-terminus corresponds to the classical CILP protein, while the C-terminus corresponds to a homologue of porcine nucleotide pyrophosphatase phosphodiesterase (NPP) (Fig. 2C and D). The CILP gene, which evolved from independent ancestral genes spanning 15.3 Kbp of genomic DNA, resides on chromosome 15q22 (43,44). Human CILP cDNA consists of 9 exons and 8 introns, of which exons 3-6 are symmetrical, while exons 7 and 8 are asymmetrical (Fig. 2A). There is a putative promoter region upstream of the encoding start site at the 5' flanking region, where regulatory elements such as GATA-1, MyoD, MZF1 and CdxA have been detected (Fig. 2B) (44). Exon 1 covers 46 bp of the noncoding region, and the eukaryotic translation of the N-terminal region corresponding to the CILP protein begins at exon 2 (43,45). Of the exons that are translated, exons 2 to 8 are 46-154 bp long, and only exon 9 exceeds 2,800 bp (44). Furthermore, exon 9 not only participates in the protein translation of CILP but also encodes the C-terminal protein, a homologue to porcine nucleotide pyrophosphohydrolase, which has piqued the interest of researchers due to its possible involvement in calcium pyrophosphate dihydrate (CPPD) deposition disease (44,46).

By means of transcriptome profiling, a homologue to CILP1 (the classical CILP) was discovered in mouse cartilage (47). During the stage before maturation, CILP2 mainly focuses on the surface of the cartilage. As maturation progresses, the homologue gradually collocates in the intermediate zone with CILP1 (47). However, there are also differences between the two analogs. In surgery-induced osteoarthritis, CILP2 was significantly downregulated, while CILP1 was upregulated. Ultrastructure analysis suggested that CILP2 may be relevant to collagen VI, which is a normal component in cartilage tissues, and that CILP2 may play a role in cartilage by mediating the interaction among the matrix components; more studies are required to test this hypothesis (47).

4. Association between cartilage intermediate layer protein (CILP) and IDD

In a 2005 study, Seki *et al* first revealed that the CILP is a key regulatory factor in IDD development (19). At the mammalian model level, Seki *et al* used transgenic mice that overexpressed CILP, and although there was no significant change in the X-ray analysis results, blood-test values or body weight, MRI analysis detected an obviously lower intensity in the area where CILP was deposited (25). In addition, through the detection and analysis of CILP content in IVDs of rabbits of different ages, it was revealed that the expression

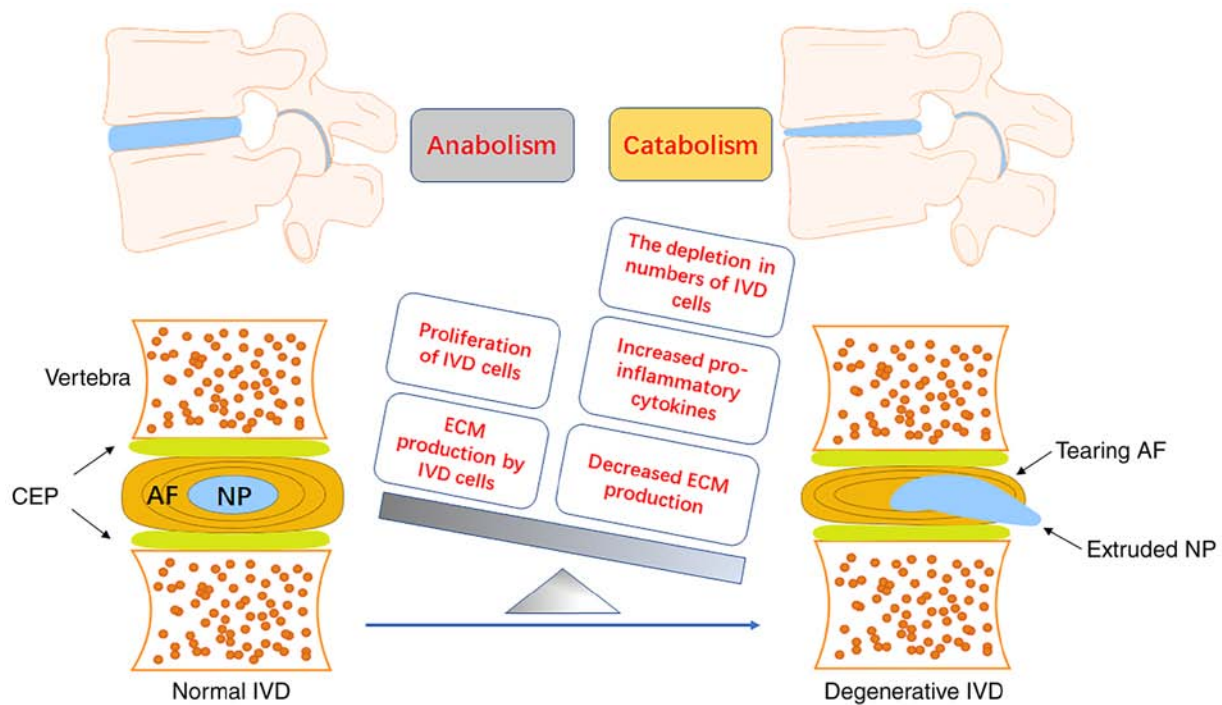


Figure 1. Development of IVD degeneration. A normal IVD is a typical sandwich structure that consists of 2 CEP, 1 AF and 1 NP. The interaction of environmental and genetic factors may lead to the degeneration of IVDs. The imbalance of anabolism and catabolism in IVD cells leads to the metabolic disturbance of the IVD microenvironment and accelerates the process of IDD. The aging or apoptosis of IVD cells reduces the production of ECM, increases the production of pro-inflammatory cytokines and ECM degradation enzymes, and promotes the catabolism of IVD cells. IVDs, intervertebral disc; CEP, cartilage endplates; AF, annulus fibrosus; NP, nucleus pulposus; IDD, intervertebral disc degeneration; ECM, extracellular matrix.

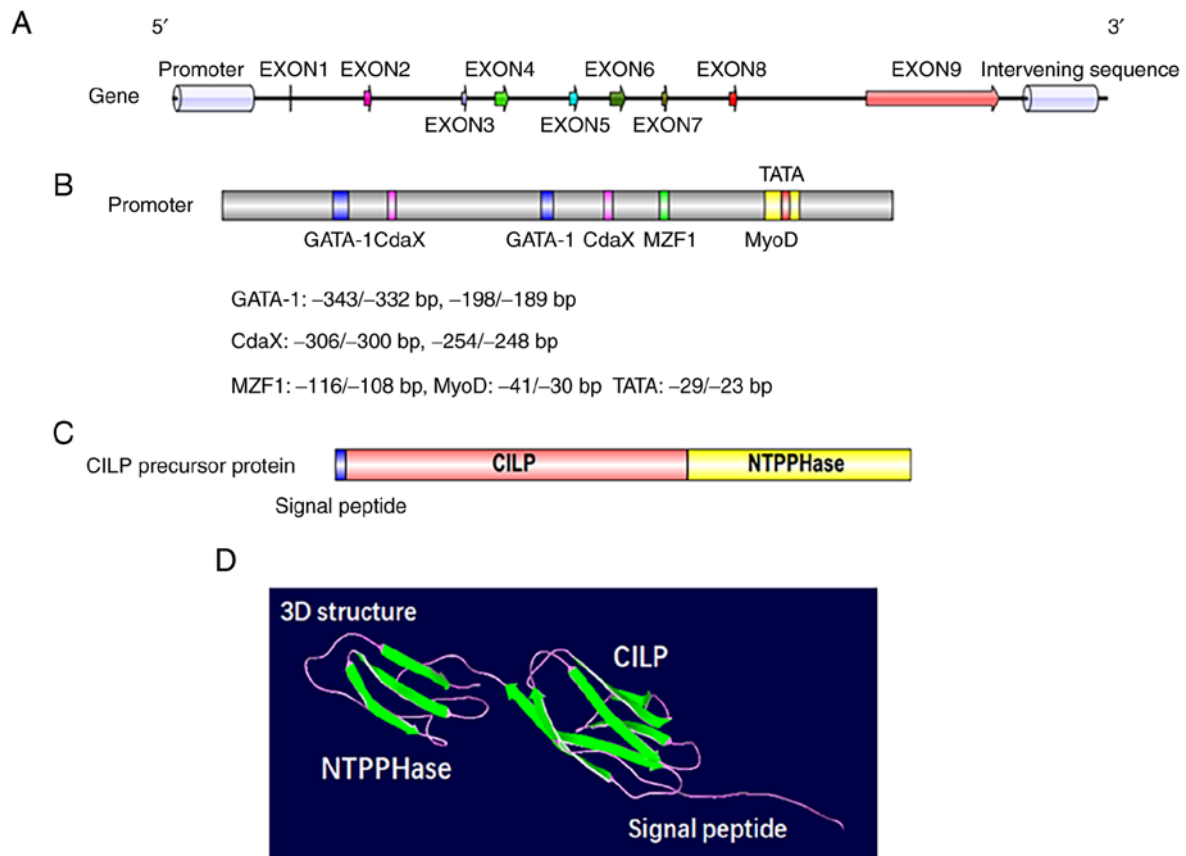


Figure 2. Genetic and protein structure of CILP in humans. (A) Genetic structure of CILP in humans. (B) Approximately 450 bp of human CILP promoter and its major regulatory binding elements, as well as factors that signal through these sites and their downstream effector factors. (C) Planar structure of a human CILP protein. (D) 3D structure of a human CILP protein. CILP, cartilage intermediate layer protein; NTPPHase, porcine 127-kDa nucleotide pyrophosphohydrolase.

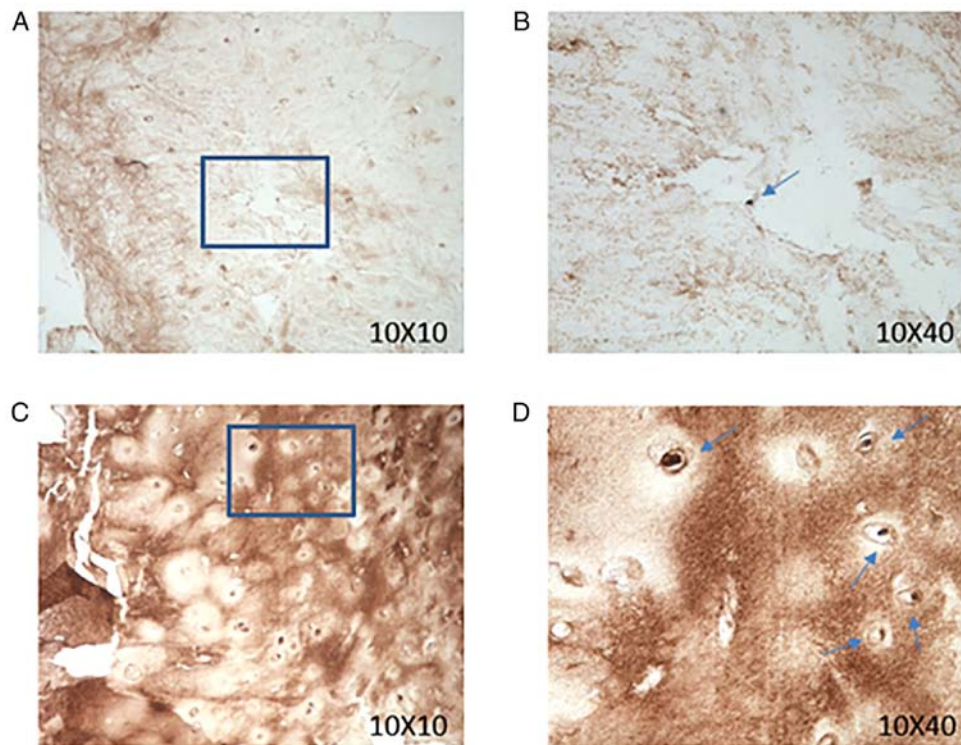


Figure 3. Expression of CILP in human intervertebral disc tissue increases with the degree of degeneration. (A and B) CILP immunohistochemical staining of Pfirrmann grade I (low degree of degeneration of intervertebral disc tissue). (C and D) CILP immunohistochemical staining of Pfirrmann grade III (high degree of degeneration of intervertebral disc tissue). Blue arrow, CILP expression positive; 10x10, zoom in 100 times; 10x40, zoom in 400 times. CILP, cartilage intermediate layer protein; NTPPHase, porcine 127-kDa nucleotide pyrophosphohydrolase.

level of CILP in IVDs in aged rabbits was significantly increased (23). Previous research has not reported CILP expression levels in human IVDs. Therefore, we collected human IVDs with varying degrees of degeneration and assessed their CILP expression levels. Human IVD tissues were collected from 18 patients (male:female, 1:1) ranging in age from 20-65 years who were undergoing lumbar spinal surgery for IDD between October 2019 and May 2020, at the Department of Orthopedics of The Second Affiliated Hospital (Chongqing, China). The present study was approved by the Ethics Committee of Xinqiao Hospital (Chongqing, China) on October 1, 2019 at the implementation of the study. All subjects provided their informed consent before participating in the present study. Immunohistochemical staining of a CILP antibody was performed in human disc paraffin sections with different degrees of degeneration in Pfirrmann grades. In brief, the tissues were fixed in 4% paraformaldehyde at 25°C for 48 h. Dehydrated tissue was immersed in xylene for 2 h for transparent treatment and then immersed in section paraffin for 2.5 h. The tissue sections were 5- μ m thick. Then, the sections were blocked in normal goat serum (cat. no. SL038; Solarbio Life Sciences) that was diluted 20 times with PBS and added directly to the slices for 10-30 min at 37°C. Tissue sections were then incubated with CILP Polyclonal Antibody (rabbit/IgG; dilution, 1:200; cat. no. PA5-51856; Thermo Fisher Scientific, Inc.) at 4°C for 12 h. Following primary incubation, the sections were incubated with HRP-conjugated goat anti-rabbit IgG (dilution, 1:100; cat. no. SA134; Solarbio Life Sciences) at room temperature (25°C) for 1 h. The sections were observed using

a light microscope (magnifications, 10 X 10 and 10 X 40). The expression of CILP was revealed to be increased in human disc paraffin sections with higher degrees of degeneration (Fig. 3). Quantitative proteomic analysis of the IVDs of different IDD level groups revealed that CILP expression was significantly increased in the IVDs of people with severe IDD, specifically, in NP tissue, there was more CILP expression in the degenerate sample (26). In recent years, genetic susceptibility analysis of diseases to obtain the degree of genetic correlation with diseases has been widely used. A study consisting of 467 Japanese patients and 654 controls revealed that the single nucleotide polymorphism (SNP) rs2073711 at the 1,184 allele (T to C) was genetically correlated with IDD (19). Furthermore, the substitution from T to C increased the binding of CILP to transforming growth factor- β (TGF- β), which enhanced the CILP-mediated suppression of the pro-anabolic effect mediated by TGF- β ; this substitution plays an important role in the pathogenesis and origin of IDD (19). A meta-analysis of genetic association studies of IVDs using a total of 1,551 IVD cases and 1,793 controls from the 5 studies which were used in this study, and comprising four Asian populations and two European populations, confirmed the positive association between the CILP gene and IVDs (48). However, the correlation was absent in a Chinese sample with 691 cases, a Finnish sample with 502 cases and an Indian sample with 342 cases (49,50). It appears that the genetic predisposition of CILP for IDD varies with population differences, which can explain the discrepancy among different ethnicities. Moreover, two studies further complicated the differential predisposition of CILP, as they revealed the genetic association between

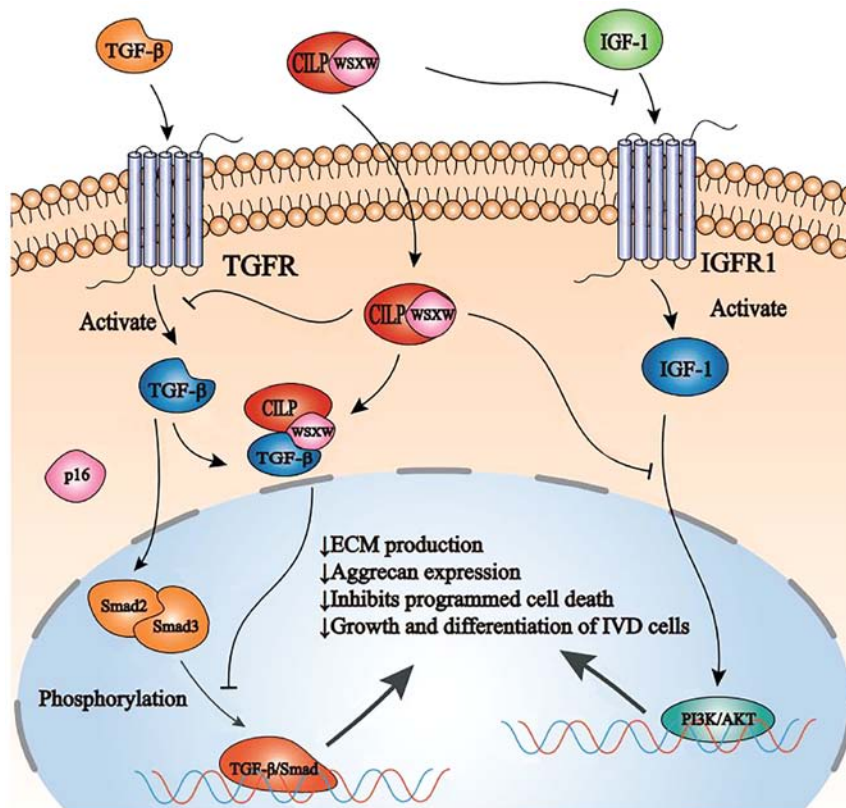


Figure 4. Schematic representation of CILP function and regulation in NP cells by TGF- β and IGF-1. CILP inhibits SMAD2/3 phosphorylation, either directly or by interfering with TGF- β binding to its receptor TGFR, ultimately inhibiting the TGF- β /SMAD signaling pathway. In addition, CILP can inhibit the binding of IGF-1 and its receptor IGF1R. Furthermore, CILP can inhibit the function of the combination of IGF-1 and IGF1R in NP cells and interfere with the PI3K/AKT signaling pathway, eventually leading to a decrease in ECM production, aggrecan expression, and growth and differentiation of intervertebral disc cells. CILP, cartilage intermediate layer protein; NP, nucleus pulposus; TGF- β , transforming growth factor- β ; IGF-1, insulin-like growth factor-1; TGFR, transforming growth factor- β receptors; SMAD, mothers against decapentaplegic homolog; PI3K, phosphatidylinositol-3 kinase; IGF1R, IGF-1 cell surface receptors; ECM, extracellular matrix.

IDD and the C allele in CILP in Japanese male collegiate athletes and judokas (42,51). In another study with a Finnish population, the rs2073711 SNP was associated with IDD among women (52). It appears reasonable that the genetic susceptibility of CILP is also gender-dependent, in addition to the existing race-dependence and the substitution from the T to the C allele that changes the character of CILP, which enhances the risk of degeneration. However, given that these athletes, especially male athletes or male judokas, experience a higher mechanical loading than that of non-athletes, this phenomenon can also be explained by mechanical overloading that leads to the odds ratio discrepancy between males and females (51). Therefore, more studies are required to provide a deeper understanding of the association between CILP and IDD.

CILP is associated with other degenerative conditions, such as osteoarthritis and myocardial fibrosis. CILP was significantly increased in articular cartilage where osteoarthritis occurred, and as a key regulatory factor, it has been revealed to play an important role in the occurrence and development of osteoarthritis (53). In recent years, CILP has been regarded as an important indicator protein for myocardial fibrosis, and its expression level indicates the severity of myocardial fibrosis and plays a positive role in clinical diagnosis; decreased levels of CILP are generally considered to indicate severe myocardial fibrosis (54).

5. Function of CILP in IDD

With the further study on the mechanism of CILP in the development of IDD, it was revealed that the expression level of CILP in NP cells has an important effect on the ECM, an important component of the extracellular microenvironment (24). Aggrecan and collagen II are the traditional degenerative markers of IDD, which are the main components of the ECM. CILP siRNA effectively inhibited CILP expression in NP cells and significantly increased the expression of aggrecan and collagen II. In addition, treatment of NP cells with a high concentration of rhCILP resulted in significantly decreased expression of aggrecan and collagen II (24). The primary function of the intervertebral disc ECM is to ensure physical and biomechanical strength (55). The ECM plays important biological roles in chondrocyte metabolism by regulating growth factors, including TGF- β (56,57). Studies have revealed that TGF- β induces the synthesis of proteoglycans and cell proliferation in IVDs (56,58). Furthermore, an injection of an adenoviral TGF- β expression vector was revealed to increase proteoglycan synthesis in human IVDs (59). The TGF- β signaling pathway is broadly involved in the growth and differentiation of cells and is responsible for the anabolism of the ECM, which is critical for the homeostasis of discs (60,61). The ECM protein decorin binds to TGF- β to form a complex that controls the accessibility of TGF- β to receptors (62,63);

Table I. Information on the relationship between CILP and IDD.

Authors	Date	Important events	(Refs.)
Lorenzo <i>et al</i>	1998	CILP is first identified and isolated	(18,20)
Lorenzo <i>et al</i>	1999	Human CILP gene is isolated and characterized	(44)
Hirose <i>et al</i>	2002	Increased CILP mRNA expression in chondrocytes promotes the formation of calcium pyrophosphate dihydrate crystals in aged cartilage	(46)
Johnson <i>et al</i>	2003	CILP is revealed to promote osteoarthritis by regulating the IGF-1/PI3K/AKT signaling pathway	(68)
Seki <i>et al</i>	2005	A functional SNP in CILP, encoding cartilage intermediate layer protein, is associated with susceptibility to lumbar disc disease	(19)
Seki <i>et al</i>	2005	CILP is revealed to promote IDD by regulating the TGF- β /SMAD signaling pathway	(19)
Virtanen <i>et al</i>	2007	Differences of CILP gene susceptibility are revealed in different populations of IDD patients	(49)
Seki <i>et al</i>	2014	CILP is revealed to promote lumbar disc degeneration in transgenic mice	(25)
Wang <i>et al</i>	2016	Association between cartilage intermediate layer protein and degeneration of intervertebral disc: A meta-analysis	(48)
He <i>et al</i>	2018	CILP is regulated by mechanical stress and affects extracellular matrix synthesis to promote the progression of IDD	(24)

CILP, cartilage intermediate layer protein; IDD, intervertebral disc degeneration.

similarly, the binding of CILP to TGF- β may interfere physically with the binding of TGF- β to its receptor, or may render TGF- β inaccessible to its receptor by sequestering TGF- β (19). CILP has a thrombospondin type 1 repeat domain that contains the WSXW motif, a well-defined consensus sequence that binds to the active form of TGF- β ; CILP coexists with TGF- β in disc tissues and the territorial matrices of TGF- β in IVDs (19). This interference results in the inhibition of phosphorylation of mothers against decapentaplegic homolog (SMAD)2/3, the key factors of the TGF- β /SMAD signaling pathway (19). Furthermore, CILP is capable of suppressing the interaction of TGF- β with its special receptor by directly binding to the growth factor, a binding that inhibits the TGF- β signaling pathway in NP cells (25). Moreover, the phosphorylation of SMAD3, a downstream effector of TGF- β , was revealed to be suppressed in transgenic mice overexpressing CILP (19,64). Alternatively, the binding of CILP may hinder the activation mechanism of TGF- β by altering its interaction with the latency complex, and may hinder the efficacy of enzymes in releasing the active form of TGF- β (19). In addition, the SNP (rs2073711) in the CILP gene increases the binding ability of CILP to TGF- β , consequently enhancing its suppression of the TGF- β signaling pathway (19). In conclusion, the overexpression of CILP upsets the balance of the control of TGF- β in chondrocyte metabolism and intervertebral disc tissue maintenance, leading to lumbar degenerative disease susceptibility caused by an inadequate response of intervertebral disc cells to injury and mechanical stress (25).

Insulin-like growth factor-1 (IGF-1) is a naturally occurring polypeptide protein hormone that plays an important role in stimulating growth during childhood and helps build and repair tissues in adults (65). In particular, IGF-1 is a key player in IVD homeostasis by upregulating both cell proliferation and the biosynthesis of ECM components in a dose-dependent manner. Once in the IVDs, IGF-1 binds to IGF-1 cell surface

receptors (IGFR1), initiating the phosphatidylinositol-3 kinase/AKT signaling pathway, stimulating cell growth and proliferation, and inhibiting programmed cell death, which leads to an increase in IVD cell population and the production of new ECM (66,67). CILP is capable of suppressing ligand-induced IGFR1 autophosphorylation and counteracting IGF-1-mediated chondrocyte proliferation and proteoglycan synthesis (68-70), thus interfering with the anabolism and catabolism of ECM, which leads to the acceleration of IDD. Other studies have reported that IGF-1 can reduce inorganic pyrophosphate (PPi), which can be generated via the alkaline nucleotide phosphodiesterase I activity of the isozymes of the NPP family (71) and is able to promote the progression of CPPD crystal deposition in aging cartilage tissues (46); in addition, CILP can affect chondrocyte IGF-1 responsiveness via N-terminal domain-mediated inhibition (68), leading to a PPi increase that can stimulate cartilage pathological calcification as CPPD crystal deposition (72). Cartilage pathological calcification can cause the degeneration of the CEP, which decreases the availability of nutrients and the exchange of metabolites, resulting in irreversible and progressive IDD (73-75).

These studies have revealed that CILP can interfere with the binding of >1 growth factor to their designated receptor, and can suppress downstream signal transduction, consequently affecting the general homeostasis of cells (Fig. 4).

6. Regulation of CILP

As CILP has been revealed to function as a contributor to IDD, it is imperative to provide insight into the regulatory mechanism underlying CILP expression. First, CILP expression is increased as age and degeneration progress (23,26); therefore, aging and degeneration are among the causes that promote CILP expression. Notably, as a structural component in the matrix secreted by chondrocytes, CILP was expressed without

the effect of SOX9 (45), which is the core transcription factor in chondrogenesis (76-78). Instead, TGF- β , another key regulator of chondrocyte differentiation and proliferation (60), is able to promote the secretion of CILP by NP cells through the SMAD and MAPK signaling pathways (45). With regard to the CILP-mediated suppression of the TGF- β signaling pathway via the binding of CILP to TGF- β (19), there appears to be a negative feedback loop between CILP and TGF- β . Similarly, bone morphogenetic protein 2 (BMP-2) also significantly increases CILP expression by increasing CILP promoter activity through the SMAD signaling pathway, an effect that increases with age (23). However, in contrast to TGF- β and BMP-2, IGF-1 downregulates CILP expression by binding to the N-terminal polypeptide domain of CILP (68). These results indicated that the regulatory effect on CILP by growth factors varies by type, and that the dysregulated secretion of growth factors may contribute to the aberrant expression of CILP. In addition, since IDD is characterized by an enhanced level of inflammatory chemokines, including IL and TNF (17,79), which have a significant influence on the secretion of NP cells, it is possible that a high level of inflammatory chemokines may regulate CILP expression. However, it was revealed CILP expression does not undergo a significant change even in a conditioned medium with a high level of IL-1, markedly increasing the expression of MMPs and ADAMTs (80). It remains unknown whether CILP is also unaffected by other inflammatory factors, and this requires further study for clarification. CILP expression is also influenced by mechanical factors; in human NP cells, CILP expression is regulated by mechanical stress, which affects the synthesis of ECM (24). In conclusion, these results suggested that, as a structural protein resident in the ECM, CILP expression is upregulated by aging and degeneration but is unaffected by IL-1; in addition, various growth factors exert different, even contrary, regulations on CILP, which in turn affect the secretion of those growth factors.

7. Conclusions and future directions

As an NP matrix protein, CILP is specifically expressed in degenerative IVD tissues, which can accelerate the progression of IDD by altering the balance of intervertebral disc matrix metabolism.

CILP is an ECM glycoprotein that is highly expressed in degenerative disc tissues and accelerates the process of disc degeneration by altering the balance of the intervertebral disc matrix metabolism (18). Since the first discovery of CILP, the regulation, expression and function of CILP have been elucidated in numerous studies (Table I). Disc degeneration is a chronic metabolic disorder of the extracellular microenvironment. Several ECM proteins, such as CILP and connective tissue growth factor (81), are involved in this process. The end result of these cytokine pathways is an imbalance of catabolism and anabolism within the disc, leading to disc degeneration, herniation, and radicular pain. A recent study has revealed that the expression and function of CILP are regulated by specific tissue and cell types (82). In intervertebral disc-related studies, CILP regulates the role of cytokines such as TGF- β and IGF-1 in IVDs (25,68,82). The TGF- β /CILP mutual regulation is important for ECM production and the two-way regulation of TGF- β and CILP (25,82). The TGF- β /SMAD axis is inhibited

by CILP eventually leading to the decrease of ECM production and aggrecan expression, as well as inhibiting programmed cell death and growth and differentiation of IVD cells (25). The activation of BMP-2 also increases CILP expression through the SMAD signaling pathway (25). In contrast to TGF- β and BMP-2, the activation of IGF-1 downregulates CILP expression, thereby inhibiting the progression of disc degeneration. IGF-1 binds to IGF1R, activating the PI3K/AKT signaling pathway, promoting cell growth and proliferation, and inhibiting programmed cell death, which leads to an increase in IVD cell population and the production of new ECM (68). In a recent study, it was revealed that mechanical changes in the disc are another important regulatory factor of CILP (24). Increased CILP expression induced by mechanical changes in the disc NP cells can promote the process of disc tissue degeneration. In the intervertebral disc, mechanical alteration is a physiological niche condition, and in order to maintain its physiological level, it may be necessary to reduce unnecessary mechanical alteration. During disc degeneration, the blood oxygen status of NP is thought to be altered due to vascular infiltration. Therefore, a reasonable reduction of TGF- β and increase of IGF-1 expression can reduce the expression level of CILP in the disc. Further research on the role of CILP in the degenerative disc and surrounding tissues is required to determine the ultimate role of CILP in this process.

In vivo and *in vitro* studies have clearly revealed that CILP affects the anabolic effects of nucleus pulposus (CMCS) on stroma production, and that it may be able to use the properties of this protein as part of a regenerative mix to treat degenerative discs. However, there have been no clinical trials of CILP-related therapy. To further investigate CILP-related therapy, understanding the relationship between CILP and tissue inflammation will be important for the successful treatment of disc disease.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

LL and JH were responsible for conceiving the study, and are the co-first authors. CLiu, MY, JF, ML, YoZ, JY and XA were responsible for literature collection and summary. YaZ, BH, CLi, YuZ and CF were responsible for reviewing and editing the manuscript. YZ and CF were responsible for supervising the study. All authors have read and approved the published version of the manuscript.

Ethics approval and consent for publication

All subjects provided their informed consent before participating in the study. The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Xinqiao Hospital (Chongqing, China) on October 1, 2019 at the implementation of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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