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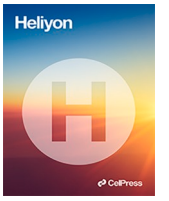

Review article

Insights into the mechanical microenvironment within the cartilaginous endplate: An emerging role in maintaining disc homeostasis and normal function

Pan Xiang [a], Zong-Ping Luo [a], Yan-Jun Che [b,*]

[a] *Department of Orthopaedics, The First Affiliated Hospital of SooChow University, Suzhou, Jiangsu, 215000, PR China*
[b] *Orthopedics and Sports Medicine Center, The Affiliated Suzhou Hospital of Nanjing Medical University, 242 Guangji Road, Suzhou, Jiangsu, 215008, PR China*

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ABSTRACT

Biomechanical factors are strongly linked with the emergence and development of intervertebral disc degeneration (IVDD). The intervertebral disc (IVD), as a unique enclosed biomechanical structure, exhibits distinct mechanical properties within its substructures. Damage to the mechanical performance of any substructure can disrupt the overall mechanical function of the IVD. Endplate degeneration serves as a significant precursor to IVDD. The endplate (EP) structure, especially the cartilaginous endplate (CEP), serves as a conduit for nutrient and metabolite transport in the IVD. It is inevitably influenced by its nutritional environment, mechanical loading, cytokines and extracellular components. Currently, reports on strategies targeting the CEP for the prevention and treatment of IVDD are scarce. This is due to two primary reasons: first, limited knowledge of the biomechanical microenvironment surrounding the degenerated CEP cells; and second, innovative biological treatment strategies, such as implanting active cells (disc or mesenchymal stem cells) or modulating natural cell activity through the addition of therapeutic factors or genes to treat IVDD often overlook a critical aspect-the restoration of the nutrient supply function and mechanical microenvironment of the endplate. Therefore, restoring the healthy structure of the CEP and maintaining a stable mechanical microenvironment within the EP are crucial for the prevention of IVDD and the repair of degenerated IVDs. We present a comprehensive literature review on the mechanical microenvironment characteristics of cartilage endplates and their associated mechanical signaling pathways. Our aim is to provide valuable insights into the development and implementation of strategies to prevent IVDD by delaying or reversing CEP degeneration.

1. Introduction

Biomechanical factors are crucial to the occurrence and development of intervertebral disc degeneration (IVDD). The endplate (EP), which is comprising both the cartilaginous endplate (CEP) and the bony endplate (BEP), plays a critical role as the attachment site for the annulus fibrosus (AF). Degeneration and damage to the EP directly weaken the mechanical integrity of the AF and alter the

* Corresponding author. Orthopedics and Sports Medicine Center, Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, 215008, Jiangsu, PR China.
E-mail addresses: xiangpan371@163.com (P. Xiang), zongping_luo@yahoo.com (Z.-P. Luo), cheyanjun1980@hotmail.com (Y.-J. Che).

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distribution of mechanical stress within the intervertebral disc (IVD), ultimately resulting in IVDD. Moreover, IVDD can be triggered by microenvironmental disturbances stemming from inflammatory factors [1], metabolic disorders [2], oxidative stress [3] and other factors. These disturbances initiate a cascade of events leading to metabolic imbalances and, ultimately, IVDD. Recently, increasing attention has been directed to the role of the CEP in the progression of IVDD, particularly in the context of the mechanical microenvironment of the EP. Therefore, gaining a deeper understanding of the biomechanics and mechanobiology of the CEP holds promise for elucidating endplate chondrocyte apoptosis and developing targeted interventions. This not only advances our comprehension of the aetiology of CEP degeneration and IVDD but also offers potential therapeutic targets. This article reviews the anatomical composition, biological functions, biomechanical microenvironment and associated mechanical signalling pathways of the CEP, thereby providing references for future research (see Fig. 1).

2. Anatomy and composition of the CEP

IVD is composed of three primary components: the nucleus pulposus (NP), AF and the endplate (BEP and CEP). The NP is a gelatinous and highly hydrated tissue rich in proteoglycans. In a healthy state, it effectively separates the adjacent vertebrae, evenly distributing compressive stress across the surrounding CEP. However, degeneration of the NP leads to disorganized fibrous tissue that significantly impairs its ability to retain water under pressure, resulting in a substantial decrease in disc height. On the other hand, the AF is an intricately organized structure composed of slanted concentric sheets of collagen fibers interspersed with proteoglycans. In cases of degraded IVDs, severe deformation occurs within the AF, potentially leading to structural damage along axial or marginal lines. The endplate serves as the connection between two adjacent vertebral bodies and plays a pivotal role in anchoring the IVD to the adjacent vertebral bone [4]. It is essential for maintaining the mechanical stability and nutrient supply of the IVD. The CEP is a thin, transparent layer of cartilage located on the surface of the BEP, positioned between the lower surface of the superior vertebral body and the upper surface of the inferior vertebral body. It is approximately 0.6 mm thick [5] and tends to thin out in its central region that interfaces with the NP. This thickness generally decreases with age. The cross-sectional dimensions of the CEP are proportional to those of the adjacent vertebral bodies. Therefore, in the cervical spine, the anterior-posterior length ranges from 16 to 19 mm, while the transverse width varies from 17 to 29 mm. In the lumbar spine, these dimensions expand to 30–36 mm in length and 43–54 mm in width. The CEP consists of approximately 60 % water, with its primary dry weight components being type II collagen and proteoglycans [6]. The collagen content is higher at the periphery of the IVD, while the proteoglycan content is lower. The three-dimensional network formed by type II collagen in the CEP inhibits the expansion of the NP and maintains a relatively stable tissue structure, which, in turn, reduces the efflux of water from the NP while facilitating nutrient diffusion from the vertebral body into the IVD. The BEP is a plate of bone that forms after the ossification process of the epiphyseal plate on both cranial and caudal sides of the vertebral body has ceased. Healthy BEP exhibit a multitude of irregular pores. As intervertebral disc degeneration progresses, the pore structure of the BEP gradually deteriorates, resulting in a rough and irregular surface. The number of "concave" pores diminishes while calcification and osteophyte formation increase. Additionally, there is a gradual reduction in pore diameter, with more pronounced changes observed in the central region compared to the peripheral region [7].

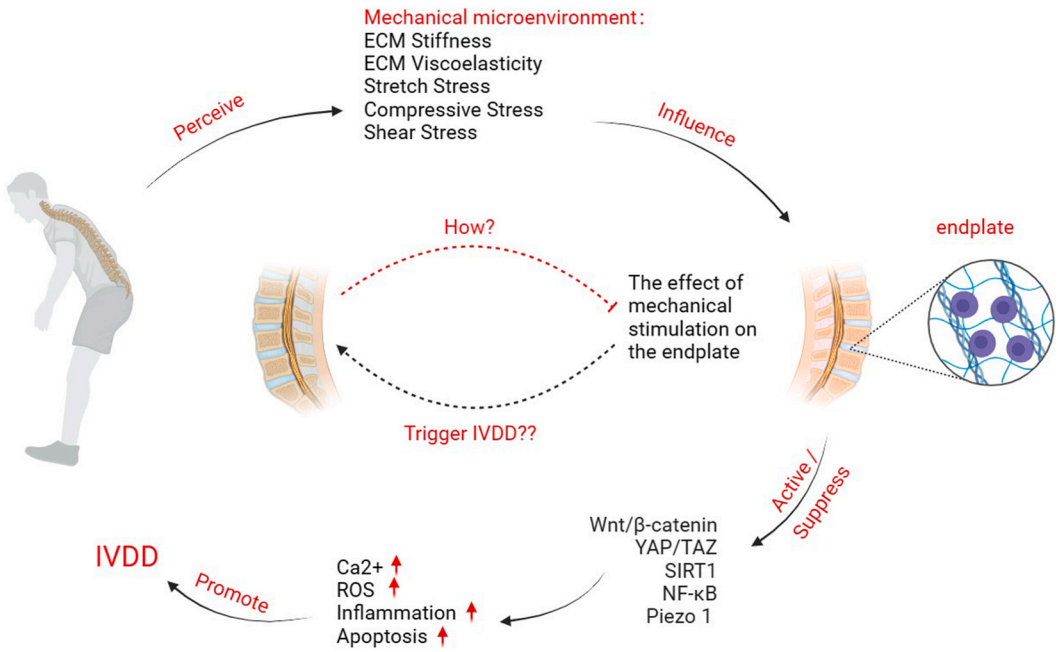

Fig. 1. Illustrates the key features of the mechanical microenvironment at the endplate during intervertebral disc degeneration.

The CEP actively synthesises extracellular matrix (ECM) components, including proteoglycans and collagen, which are vital for its physiological functions and structural integrity. The columnar arrangement of the CEP makes it well-suited for bearing compressive stress. Proteoglycans and collagen are the primary constituents of the ECM in the CEP, playing crucial roles in maintaining its physiological functions. Studies have revealed that the proteoglycan composition in the CEP is similar to that of articular cartilage. The net negative charge carried by proteoglycans governs the distribution of charged solutes and osmotic pressure within the matrix, resulting in higher ion concentration than plasma and maintaining high osmotic pressure. An abundance of proteoglycans correlates with higher osmotic pressure [8,9]. Under physiological conditions, proteoglycan aggregation levels fluctuate over time; however, in general, they decrease with increased age and prolonged exposure to excessive mechanical load, especially in the peripheral deep and central regions of the CEP. These changes directly impact the transport, absorption oscillation, and uniform transmission of loads within the CEP [10].

Water content in different substructures of the IVD depends on the ratio of proteoglycans to dry weight and the applied load exerted. Regions with a higher proportion of proteoglycans exhibit a higher level of hydration, a critical factor in enabling the CEP to resist mechanical loads. Additionally, various types of collagen proteins are transported through the CEP. The microfibers of these collagens are arranged parallel to the upper and lower surfaces of the vertebral body, with proteoglycan molecules attached. The cross-linking of the microfibers creates numerous spaces, several thousand micrometres in size, forming a highly permeable elastic network.

Type II collagen is the most abundant type of collagen in the CEP, along with type IX collagen and 1α, 2α and 3α chains. Under long-term mechanical stress, lysosomal enzymes in the IVD substructure cells are released, activating the collagenase system. Type II collagen demonstrates significant resistance to collagenases compared to other types of collagen, such as type I and type III collagen, particularly as physiological degeneration occurs. This collagen composition in the CEP ensures its ability to withstand high forces and frequent permeation exchange, thereby maintaining structural stability and facilitating physiological functions. Additionally, type II collagen is the most efficient in absorbing and supporting compressive stress, providing essential structural support for the biomechanical performance of the CEP.

3. Mechanical microenvironment of the CEP

The cellular mechanical microenvironment encompasses the conditions in which cells exist, including neighbouring cells, ECM (proteins, polysaccharides and other macromolecules) and various physical and biochemical factors. Maintaining a dynamic equilibrium within this microenvironment is essential for normal cell proliferation, migration and differentiation. Previous studies have predominantly focused on the biochemical aspects of this microenvironment, such as ECM, body fluids and growth factors, often overlooking the presence of mechanical factors. However, with advancements in biomaterials, biofabrication and characterisation technologies, there has been a growing recognition of the significance of the cellular mechanical microenvironment (matrix stiffness and stress/strain) [11,12]. In the cellular mechanical microenvironment, ECM stiffness (passive stimulation) and stress/strain load (active stimulation) are the two primary components. This section discusses the effects of the mechanical microenvironment on the cellular activities of the endplate cartilage cells, focusing on four aspects: ECM stiffness, viscoelasticity, tensile and compressive stress and fluid shear force.

3.1. ECM stiffness

The stiffness of the ECM surrounding cells varies across different tissue types. For example, soft tissues such as fat and nerves exhibit ECM stiffness ranging from 100 to 50,000 Pa [13], while in adult animals, teeth and bone tissues can reach levels in the MPa. Researchers have observed that mesenchymal stem cells cultured on matrices with differing stiffness levels tend to differentiate into distinct cell types based on matrix stiffness [14,15]. Similarly, different matrix stiffness levels have varying effects on the cellular activities of the CEP [16]. Researchers have used polyacrylamide gels with varying stiffnesses to investigate the physiological differences of the CEP on matrices of different stiffness levels. Experimental findings have revealed significant stiffness-dependent differences in the morphology, cytoskeletal organisation and proliferation of CEP chondrocytes. Moreover, elevated matrix stiffness has been associated with increased cell spreading and proliferation. The cellular response to matrix stiffness is mediated through the formation of stress fibres within the cells, leading to an increase in cell area and aspect ratio. Matrix stiffness can affect the expression of microRNA (miRNA) in endplate cartilage cells. The co-expression network analysis of miRNA and mRNA microarrays has revealed that elevated miR-20a levels result in increased calcium deposition in the CEP, ultimately inducing CEP degeneration. Therefore, an escalation in matrix stiffness can result in heightened miR-20a levels in CEP cells, culminating in CEP calcification and subsequent IVDD [2]. However, a suitable in vivo model that accurately demonstrates the impact of alterations in the extracellular matrix on CEP remains elusive.

3.2. ECM viscoelasticity

In the body tissues, the ECM enveloping cells predominantly exist in a gel-like state, comprising over 90 % water. It exhibits both flow and elastic properties under stress, with these mechanical characteristics evolving. Following the removal of external forces, it partially regains its shape, exhibiting viscoelastic behaviour. Viscoelasticity includes aspects such as stress relaxation rate, creep, loss modulus and plasticity. Traditionally, the ECM was regarded as a linear elastic material, overlooking the influence of ECM viscoelasticity on cellular behaviour. However, recent research has highlighted the pivotal role of ECM viscoelasticity in cell adhesion, spreading and differentiation (citation). Studies have revealed that ECM stress relaxation significantly affects the adhesion and

spreading of fibroblasts [17]. Other studies have demonstrated that changes in ECM stress relaxation, loss modulus, creep, and plasticity regulate cell diffusion [18], focal adhesion growth, stress fibre formation [19], and YAP nuclear translocation [17]. The increase in the loss modulus of polyacrylamide (PAM) gel was found to enhance the diffusion area and proliferation of hMSCs, while simultaneously reducing the size and maturity of the adhesive patch. Moreover, hMSCs exhibited an augmented capacity for differentiation into multiple lineages on substrates characterized by a high loss modulus [20]. While direct evidence regarding the influence of matrix viscoelasticity on the physiological activities of chondrocytes in the growth plate is currently lacking, it is noteworthy that the entire IVD possesses viscoelastic properties. In tissues such as cartilage, viscoelasticity plays a pivotal role in their mechanical functionality by influencing the fluid exchange within the tissue. The permeability of cartilage significantly impacts the transportation of nutrients and waste across cell membranes. Under mechanical loading, viscoelastic behavior dynamically alters the exudation and reabsorption of fluids, thereby modulating the tissue's permeability. Under compressive stress, the creep of the IVD increased with stress levels. Finite element modelling studies have indicated that during the creep process, fluid influx and efflux primarily occur through the highly permeable CEP. In cases of IVDD, the permeability of the CEP diminishes, leading to reduced fluid flow capacity within the intervertebral disc. This significantly impacts the inward flow of fluid following stress loading, hampering the restoration of IVD height [21]. These research findings underscore the significant impact of decreased CEP permeability on the creep characteristics of the intervertebral disc during IVDD.

3.3. Tensile and compressive stress

Cells in the body not only sense the mechanical properties of the ECM but are also exposed to complex stress stimuli within their microenvironment. Two prominent types of stress include tensile and compressive stresses. Nearly all connective tissues experience some degree of tensile stress, while compressive stress is predominant in bone tissue. Tensile stress plays a crucial role in regulating cell behaviour and ECM remodeling, among other physiological activities. In vitro experiments have applied intermittent cyclic mechanical tension to endplate cells to investigate the effects of tensile stress on their physiological activities. This tension induces significant changes in the F-actin cytoskeleton of endplate cells, transitioning their morphology from polygonal to spindle-shaped [22]. Regarding cellular vitality, short-term moderate tension stimulation helps maintain the stable phenotype and characteristics of endplate cells. In contrast, prolonged tension stimulation can induce degeneration and calcification of endplate cartilage, while it does not impact the viability of endplate chondrocytes during the early stage of stimulation [23]. Instead, it triggers degenerative changes through other signalling pathways. For example, intermittent cyclic tension loading leads to the reduced expression of COL-2A, ACAN and Sox9 in endplate cartilage cells [24]. Proteoglycans are essential for the biomechanics of cartilaginous endplates, forming the biomechanical foundation for them. Consequently, alternations in proteoglycan content and composition can affect cartilaginous endplate function, thereby influencing IVD stability and potentially leading to IVDD. Tensile stress not only reduces cellular components but also stimulates the production of inflammatory factors in endplate cartilage cells. For instance, intermittent cyclic tension loading increases the expression of MMP3, MMP9, MMP13, iNOS and COX-2 in endplate cartilage cells [25]. Intermittent cyclic mechanical tension promotes endplate cartilage degeneration through the classical Wnt signalling pathway and E-cadherin/β-catenin complex [26]. Similarly, intermittent cyclic pressure loading causes morphological changes in endplate cells from polygonal to elongated. Over time, the expression levels of type II collagen, aggrecan and SOX-9 in endplate cells decrease, indicating a time-dependent downregulation. Conversely, the expression levels of type I collagen, type X collagen and osteocalcin increase. This demonstrates that intermittent cyclic pressure directly induces the degeneration of the endplate cartilage cells, leading to the downregulation of cartilage marker genes, such as type II collagen, proteoglycans and SOX-9, and the upregulation of osteogenic marker genes, such as type I collagen, type X collagen and osteocalcin [27]. Similarly, inappropriate stress also affects the physiological activities of the CEP. Furthermore, researchers applied pressure to IVD samples through external fixation and found that the IVD exhibited varying degrees of degeneration [28]: CEP calcification increased, ACAN and type II collagen production decreased, BEP porosity decreased and bone spur formation increased [29–31]. It is widely acknowledged that the type, size, frequency, and duration of the load play a crucial role in determining cellular response [32,33]. Moderate loading at low frequencies on intervertebral disc cells has been shown to promote anabolism, whereas heavy loads with high frequencies and continuous static loading can induce catabolism or counteract anabolism. Our study validates that moderately controlled axial traction (tensile stress) disrupts the cascade of disc degeneration caused by axial compressive stress [31]. Degenerative discs exhibit significant positive remodeling following low-tension traction. It is evident that the mechanical microenvironment of the disc relies more on low-tension traction rather than high-tension traction [34,35].

3.4. Shear stress

In addition to axial loads, bones also experience transverse shear loads, leading to shear deformation across bone cross-sections. Moreover, fluid shear forces play a crucial role in bone growth. The cancellous bone features a porous scaffold comprising osteons, bone lacunae and trabeculae, which are filled with tissue fluid. When bones bear loads, the unmineralised matrix surrounding cells compresses, resulting in fluid shear stress on the bone cell membrane. This process regulates the proliferation of cells and the synthesis and secretion of BMP and TGF-β, thereby promoting bone growth. Various physical activities generate varying degrees of shear stress. Tests on human IVD samples have identified the regions of maximum shear strain experienced during axial compression, axial rotation, anterior-posterior shear, flexion/extension, lateral shear and lateral bending. Among these, lateral bending and flexion induce the maximum shearing motion [36]. Finite element analysis revealed that for all loads, the maximum shear force strain occurs between the AF and the CEP [37]. Similarly, in IVD samples, the maximum tensile strain and shear strain peaks are near the endplate [38].

Excessive shear loads have the potential to induce IVDD. Prolonged shear loading can lead to IVD dysfunction, resulting in lumbar instability and progressive IVDD [39].

4. Cartilaginous endplate and mechanical signalling pathways

Previous findings in this study have demonstrated that inappropriate mechanical loading can contribute to the occurrence or worsening of disc degeneration. Abnormal stress (such as overload and external fixation) can expedite disc degeneration and facilitate the calcification and degeneration of CEP. To our knowledge, there is a lack of comprehensive reports summarizing the mechanism behind CEP degeneration caused by mechanical stress. Due to limited relevant studies, this paper primarily focuses on discussing several mechanosensitive pathways involved in mechanical stress-induced CEP degeneration, including Wnt/β-catenin, YAP/TAZ, SIRT1, and other classical mechanosensitive pathways. We not only describe the impact of these pathways on the physiological activity of CEP but also emphasize how mechanical stress affects CEP through these pathways. By considering the mechanical microenvironment of CEP as a starting point, we further elaborate on molecular targets within CEP and highlight potential mechanisms underlying CEP degeneration induced by mechanical stress (Table 1).

4.1. Wnt/β-catenin signalling pathway

The Wnt signalling pathway plays a crucial role in regulating the development of various tissues and organs, the change of which is often implicated in human diseases. The canonical Wnt pathway, known as the Wnt/β-catenin signalling pathway, becomes activated when Wnt proteins bind to a receptor complex consisting of Frizzled receptors and low-density lipoprotein receptor-related proteins 5/6 [67]. In the absence of Wnt ligands, β-catenin undergoes phosphorylation of glycogen synthase kinase 3 (GSK3) and is targeted for ubiquitin-dependent proteolysis. Conversely, in the presence of Wnt ligands, GSK3 activity is inhibited, leading to the accumulation and nuclear translocation of β-catenin [68]. In the cell nucleus, β-catenin binds to T-cell factor/lymphoid enhancer factor (TCF/LEF) to activate target gene promoters [69]. Therefore, β-catenin is a key player in the canonical Wnt signalling pathway. Importantly, Wnt/β-catenin can sense extracellular mechanical stimulation and contribute to CEP homeostasis and degeneration [40]. Intermittent cyclic tensile loading activates the Wnt/β-catenin pathway in endplate cartilage, resulting in the reduced expression of COL-2A, ACAN and Sox9, thereby promoting calcification [24]. Elevated β-catenin expression has also been observed in degenerated IVD tissues, and its activation further exacerbated disc damage [41,42]. Additionally, the application of Licl, a β-catenin activator, to CEP cartilage leads to increased cell apoptosis, decreased cell viability, and an increased number of senescence-associated β-galactosidase (Sa-β-gal) positive cells, ultimately promoting CEP degeneration. After treatment with Licl, the expression of MMP13 and ADAMT-5 in CEP cells increased, whereas the expression of proteoglycans decreased [40]. Rac-1, a GTPase of the Rho family, is instrumental in cartilage differentiation [43] and can control β-catenin phosphorylation and nuclear localisation [70], further enhancing its interaction with LEF-1 [44]. In degenerated CEP, Rac-1 expression significantly rises. After treatment with NSC23766 (a Rac-1 inhibitor), degenerated CEP exhibited upregulated expression of anabolic genes such as type II collagen and ACAN and decreased expression of catabolic genes such as MMP13 and ADAMT-5. Thus, these findings demonstrate that inhibiting Rac-1 activity with NSC23766 prevents CEP degeneration through the Wnt/β-catenin pathway [45].

The Wnt/β-catenin signaling pathway exerts regulatory control over the proliferation and differentiation of CEP cells, as well as the synthesis of essential cartilage matrix proteins (collagen and proteoglycans). Moreover, it indirectly influences the health and degeneration of CEP by modulating inflammatory cell activity and cytokine levels. Given its pivotal role in various degenerative and inflammatory diseases, targeting this pathway with inhibitors holds promise for treating CEP degeneration. For instance, blocking Wnt signaling using Dkk-1 (Dickkopf-1), a natural inhibitor of this pathway, or other molecular inhibitors may effectively slow down the degenerative process. In summary, the intricate involvement of the Wnt/β-catenin signaling pathway in maintaining or deteriorating CEP health necessitates further research to fully comprehend its underlying mechanisms; however, existing studies suggest multiple

Table 1
Summary of mechanical signalling pathway.

Mechanical signaling pathway	Mechanical stimulation	Effect on endplate	Reference
Wnt/β-catenin YAP/TAZ	ICMT/Compress stress ICMT/ECM Stiffness	The Wnt/β-catenin pathway in the endplate cartilage is activated after intermittent cyclic tension loading, leading to a decrease in the expression of COL-2A, ACAN, and Sox9 in the endplate cartilage and promoting calcification. YAP/TAZ not only perceive alterations in cellular morphology and cytoskeletal tension, but also exhibit responsiveness towards diverse ECM stiffness.	[24,40–48]
SIRT1	ICMT	After melatonin treatment, the expression of SIRT1 in the cartilage endplate significantly increased, and the apoptosis and calcification of the cartilage endplate were prevented through the SIRT1-autophagy pathway.	[49–53]
NF-κB	ICMT	CGA and melatonin can inhibit the NF-κB signaling pathway, protecting the cartilage endplate from inflammation, catabolism, apoptosis, etc., thereby delaying the progression of disc degeneration.	[54–57,57,58]
Piezo1	ICMT/ECM Stiffness	Under the action of ICMT, the increased expression of Piezo1 induces the influx of Ca2+, leading to YAP pathway activation and an increase in the expression of inflammatory factors.	[59–66]

ICMT: Intermittent Cyclic Mechanical Tension.

potential therapeutic targets within this pathway that could lead to novel strategies for managing related degenerative diseases. Rigorous investigation through clinical trials is warranted to validate both efficacy and safety profiles associated with these potential therapeutic interventions.

4.2. YAP/TAZ signalling pathway

YAP/TAZ serves as primary sensors of cellular mechanical properties, which are determined by cell structure, shape and polarity. YAP/TAZ activation is reflective of cellular 'social' behaviours related to cell adhesion and response to mechanical signals received from tissue structure and the surrounding ECM. Additionally, YAP/TAZ is associated with morphogenic signals such as Wnt growth factors. YAP/TAZ can sense changes in cell shape, cytoskeletal tension and variations in ECM stiffness [46,47]. Oncostatin M (OSM), a protein secreted by bone tissue macrophages, promotes YAP1 phosphorylation, interaction and nuclear translocation with STAT3, resulting in osteogenic differentiation. Blocking OSM has been found to rescue CEP calcification, indicating OSM-induced CEP calcification through STAT3/YAP1 phosphorylation and nuclear translocation [48].

The YAP/TAZ pathway plays a pivotal role in the regulation of cartilage cell proliferation and differentiation, thereby influencing the cellular composition of CEP. In healthy CEP, this pathway contributes to maintaining cellular renewal and repair capabilities. However, under pathological conditions, aberrant activation of YAP/TAZ may promote the growth of atypical cells or dysregulation of differentiation. During CEP degeneration, imbalanced remodeling and destruction of the ECM emerge as key factors leading to dysfunction. By modulating the expression of matrix molecules such as collagen and proteoglycans, YAP/TAZ can impact the structural integrity of the endplate. Moreover, it is suggested that YAP/TAZ might exacerbate or alleviate inflammation by regulating immune cell infiltration and release of inflammatory mediators during CEP degeneration. Targeting upstream signals (e.g., components within Hippo signaling pathway) or downstream effects (e.g., interactions with transcription factors) associated with the YAP/TAZ pathway represents potential therapeutic strategies. Precise modulation rather than complete inhibition could be achieved by finely adjusting these signals to regulate YAP/TAZ activity within cells. A comprehensive understanding regarding how precisely YAP/TAZ influences CEP cell behavior will facilitate development of more precise and effective therapeutic interventions.

4.3. SIRT1 signalling pathway

Sirtuins are a conserved family of NAD + -dependent deacetylases and ADP-ribosyl transferases. SIRT1 is located in the cell nucleus and cytoplasm, exerting its effects by deacetylating substrates involved in metabolism and ageing regulation. SIRT1 is closely associated with osteogenic differentiation [71,72]. For example, mechanical stretching activates the AMP-induced antioxidant response and osteogenic differentiation-activating protein kinase-SIRT1 signalling pathway in human mesenchymal stem cells [49]. SIRT1 overexpression was reported to enhance osteoblast bone formation and prevent bone loss, thus ameliorating skeletal defects. For cartilaginous endplates, SIRT1 not only inhibits apoptosis and calcification induced by oxidative stress but also prevents CEP senescence.

Melatonin, an endocrine hormone synthesised by the pineal gland, regulates the circadian rhythm [73]. Studies also report an association between melatonin and IVDD. For example, the removal of the pineal gland in chickens promoted the progression of IVDD [50]. Studies have shown that melatonin downregulates MMP3/9 expression in IVD cells and upregulates COL2A1 and ACAN expression [51]. Additionally, melatonin treatment increases SIRT1 expression in CEP cells, preventing apoptosis and calcification through the SIRT1-autophagy pathway. Moreover, the expression of Bax, RUNX2 and OCN in the CEP decreases, whereas that of Bcl-2 increases. These effects are significantly attenuated after intervention with a SIRT1 inhibitor [52]. Furthermore, SIRT1 exerts anti-cellular senescence effects. On injecting Adipo-sEVs (adipose-derived small extracellular vesicles) into senescent CEP, researchers observed that Adipo-sEVs promoted the recovery of senescent CEP by delivering NAMPT and activating the SIRT pathway, enhancing the synthesis of NAD+ and leading to the decreased expression of calcification-related genes RUNX2 and OCN [74].

SIRT1 exerts its anti-inflammatory effects in CEP by deacetylating key transcription factors, such as NF-κB, thereby reducing the production of inflammatory cytokines. This mechanism attenuates inflammation responses induced by mechanical stress or biochemical factors and downregulates the expression of inflammatory mediators like IL-1β and TNF-α, ultimately safeguarding CEP against further degeneration. Moreover, SIRT1 plays a pivotal role in regulating energy metabolism and counteracting oxidative stress. In CEP cells, SIRT1 activity maintains cellular energy homeostasis and enhances resistance to oxidative stress, consequently delaying cellular aging and degenerative processes. Additionally, SIRT1 may also participate in modulating the synthesis and degradation of the cartilage matrix. Research findings indicate that SIRT1 can impede collagen degradation by influencing the activity of MMPs, thereby promoting the structural integrity of CEP. SIRT1 activators, such as resveratrol and SRT1720, have demonstrated anti-inflammatory and anti-aging effects in diverse cellular and animal models. The application of these activators may augment the maintenance and repair capabilities of CEP, decelerating or reversing the degeneration process. In clinical applications, although modulating SIRT1 presents a promising approach for treating CEP degeneration, further research and clinical trials are necessary to validate its potential long-term effects and possible side effects. Specifically, precise control over increased SIRT1 activity and understanding the specific impacts of this regulation on different patients at various stages of the disease represent crucial directions for future investigation.

4.4. NF-κB signalling pathway

The NF-κB transcription factor family plays a pivotal role in mediating cellular responses to injury, stress and inflammation. Several pro-inflammatory cytokines and mediators are regulated by NF-κB, such as tumour necrosis factor α, interleukin-1 and interleukin-6

[75]. The mammalian NF-κB family comprises five subunits, with the p50/p65 heterodimer being the most common and abundant form.

NF-κB signalling can be activated by extracellular mechanical signals. Intermittent cyclic tension, for instance, activates the NF-κB pathway in CEP cells, leading to the increased expression of NF-κB and pro-inflammatory factors, ultimately causing CEP inflammation and contributing to IVD disease [54]. The overexpression of P120-catenin inhibits NF-κB signalling transduction, mitigating intermittent cyclic tension-induced CEP cell inflammation [25]. Similarly, reactive oxygen species (ROS) also affect NF-κB signalling pathway activity. Treatment with H_2O_2 increases intracellular ROS levels in CEP cells along with the time-dependent elevation of phosphorylated ERK, P38 and P65, thus promoting the apoptosis and calcification of CEP cells [55]. Chlorogenic acid (CGA), a natural bioactive compound extracted from herbs such as *Eucommia* and *Lonicera japonica*, has immunoprotective, analgesic [76], anti-apoptotic, antioxidant and anti-inflammatory properties [77]. Compounds like CGA and melatonin [78] can protect the CEP from inflammation, catabolism and apoptosis by inhibiting the NF-κB signalling pathway, thereby delaying the progression of IVD degeneration [56]. Conversely, Propionibacterium acnes promotes CEP degeneration by enhancing macrophage migration inhibitory factor (MIF) expression through the NF-κB pathway [57]. The division of macrophages into pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes, based on the expression of cytokines and functions, allows the latter to facilitate tissue repair by mitigating inflammation [79]. Consequently, inhibiting MIF expression can impede the inflammatory cascade associated with CEP degeneration caused by the release of inflammatory factors such as IL-1β, TNF-α, IL-1α, IL-2, IL-4, IL-6 and prostaglandin E2. Thus, the regulation of the NF-κB pathway can significantly impact the expression of pro-inflammatory factors in CEP cells, thereby influencing their cellular activities.

NF-κB, a pivotal transcription factor governing inflammatory responses, can be activated in the degeneration of CEP due to mechanical stress, biochemical factors, or cellular damage. Consequently, it orchestrates the expression of diverse inflammation-related genes encompassing cytokines (e.g., TNF-α and IL-1β), chemokines, and adenylate cyclase inhibitors. The upregulation of these inflammatory mediators exacerbates CEP deterioration by hastening the degenerative process. Additionally, NF-κB plays a crucial role in promoting cell survival and inhibiting programmed cell death (apoptosis). In certain cases, this mechanism can contribute to the preservation of cartilage cell viability; however, excessive or inappropriate activation may lead to pathological alterations. Direct inhibitors targeting NF-κB activity, such as IKK inhibitors (IκB kinase inhibitors) or small molecule inhibitors that specifically target NF-κB subunits, hold promise for alleviating inflammatory responses and delaying CEP degeneration. Suppression of upstream signals activating the NF-κB pathway, including those from Toll-like receptors (TLRs), TNF, or ILs, can effectively reduce NF-κB activation. The utilization of TNF inhibitors (e.g., infliximab or adalimumab) or IL-1 receptor antagonists has demonstrated efficacy in other inflammatory diseases. These potential therapeutic strategies offer hope for developing novel treatment plans aimed at mitigating pathological changes and symptoms associated with CEP degeneration. However, further research and validation are still required before these strategies can be clinically applied, particularly regarding their preventive effects during early disease stages and their effectiveness within complex biological environments.

4.5. Piezo ion channels

Piezo is a cation channel protein that directly senses mechanical force on the membrane, enabling ion passage. Vertebrates have two types of Piezo proteins: Piezo1 and Piezo2. Piezo1 plays pivotal roles in various physiological processes, such as sensing blood flow shear stress to facilitate vascular formation, regulating red blood cell function and controlling cell migration and differentiation. Both Piezo1 and Piezo2 are mechanically activated ion channels that can detect changes in mechanical stress and convert them into electrical or chemical signals [59,60]. They are widely distributed in various tissues, such as bone, articular cartilage and the IVD [61,62]. Piezo1 is extensively present in IVD cells and holds significant importance for the physiological activities of various IVD components. In NP cells, mechanical stimulation increases Piezo1 protein expression. Moreover, Piezo1 activates the NF-κB signalling pathway by increasing intracellular Ca2+ influx, resulting in the activation of NLRP3 inflammasomes in NP cells [80,81], ultimately leading to the apoptosis of NP cells. Furthermore, it has been demonstrated that the Nrf2/TXNIP/NLRP3 axis confers protection to MSCs against the detrimental effects of reactive oxygen species microenvironment, thereby facilitating disc repair [82]. Additionally, Piezo1 can influence the autophagy level in NP cells, further impacting IVD tissue [83]. Indeed, numerous studies have demonstrated that the upregulation of Piezo1 channel proteins significantly enhances the differentiation of adipose-derived mesenchymal stem cells into nucleus pulposus -like under increased mechanical stimulation in vitro. Furthermore, in vivo experiments have revealed a notable increase in disc height and extracellular matrix content within the NP region [84]. For AF cells, abnormal mechanical loading induces AF cell apoptosis and exacerbates IVDD through the activation of Piezo1 and the downstream Calpain2/BAX/Caspase3 pathway [85]. Similarly, in CEP cells, increased Piezo1 expression under intermittent cyclic tensile stress induces Ca2+ influx, activates the YAP pathway, increases inflammatory factor expression and contributes to degeneration, thereby exacerbating IVDD [63,64]. Various evidence suggests that Piezo1 may represent a potential therapeutic target for IVDD treatment. This study has some limitations. The main reason is that so far, there are few studies on the mechanical microenvironment of cartilage endplate. Although we use different databases to search, there are still some omisations.

Piezo1, functioning as a mechanosensitive channel, directly senses mechanical stress in CEP cells. Its activation induces the influx of calcium ions into the cell, thereby initiating a cascade of downstream signaling pathways involved in cellular proliferation, differentiation, and matrix synthesis and degradation. Additionally, Piezo1 activation modulates gene expression associated with ECM synthesis and degradation, including MMPs and TIMPs, which are pivotal for maintaining structural integrity and functional homeostasis of CEP. Furthermore, Piezo1 channel activation may also regulate inflammatory responses. Mechanical stress and calcium signaling induced by Piezo1 can modulate the production of inflammatory cytokines, thereby influencing the inflammatory state of

CEP. Utilizing Piezo1 inhibitors presents a potential strategy for mitigating degeneration caused by excessive mechanical pressure. For instance, blockade of Piezo1 could attenuate aberrant mechanotransduction, thus preventing cellular and matrix damage resulting from excessive pressure. Given the multifaceted roles played by the Piezo1 pathway in CEP degeneration, further investigation and validation of this channel are warranted. Specifically, elucidating the precise mechanisms underlying Piezo1-mediated mechanotransduction and exploring how modulation of this channel can optimize physiological responses in CEP represent crucial areas for future research.

5. Conclusion

IVDD is a cascade reaction triggered by alterations in the mechanical environment, leading to cell-mediated biochemical, mechanical, and structural changes. As a dynamic mechanical barrier of the IVD, the endplate's biomechanical properties are determined by the composition of its ECM. The capacity to endure mechanical forces hinges on the structural integrity of the matrix framework. Among the substructures of the IVD, the CEP, in particular, is susceptible to degeneration under abnormal stress conditions, occurring either prior to or concurrently with degeneration of the NP and AF. This ultimately leads to impaired disc function, which involves an imbalance between ECM synthesis and degradation, where the elasticity and stiffness influence cellular stretching, growth, proliferation, migration, and differentiation. Although these changes also occur during normal aging, IVDD accelerates their occurrence and progression. This, in turn, self-perpetuates under abnormal stress conditions, leading to further damage to disc function. Therefore, a stable endplate mechanical microenvironment based on the restoration of healthy CEP structure will provide a new strategy for clinical prevention of IVDD and repair of degenerative IVD.

6. Prospect (clinical transformation and application)

In the context of clinical research on IVDD and CEP degeneration, understanding the behavior of CEP cells and their response to changes in the mechanical microenvironment is of paramount importance. These cells are subjected to shear, compressive, and tensile stresses that directly impact both the cells themselves and the surrounding extracellular matrix, thereby influencing its stiffness and overall structural stability. Consequently, comprehending and regulating the effects of these mechanical stresses on cellular function is crucial for advancing therapeutic strategies.

In the field of clinical treatment research and applications, the modulation of signaling pathways such as Wnt/β-catenin, YAP/TAZ, SIRT1, NF-κB, and Piezo1 has demonstrated promising therapeutic potential. These pathways play crucial roles in regulating cell survival, proliferation, differentiation, and response to mechanical stress. They serve as vital links between fundamental biological research and clinical applications. Specifically: manipulation of the Wnt/β-catenin pathway can enhance the proliferation and differentiation of CEP cells while promoting extracellular matrix synthesis to maintain IVD structural integrity; activation of the YAP/TAZ signal is closely associated with the mechanical microenvironment and can enhance cellular mechanosensitivity and adaptability by modulating cell-matrix interactions; activation of SIRT1 confers protection against oxidative stress and inflammation, thereby delaying degenerative processes and improving cellular homeostasis; inhibition of the NF-κB pathway can attenuate inflammatory mediator production to prevent excessive inflammatory responses that exacerbate disc damage; investigation into the Piezo1 channel has unveiled its pivotal role in mechanotransduction-mediated cellular functions, offering novel approaches for regulating cellular responses to mechanical stimuli.

In the realm of clinical treatments for managing IVD and CEP degeneration, numerous therapeutic approaches have been developed and explored with the aim of fundamentally altering the progression of these degenerative diseases. These methods capitalize on a profound understanding of the aforementioned signaling pathways and strive to offer more targeted and enduring interventions through molecular-level manipulations. Key therapeutic strategies encompass.

6.1. Molecular targeted therapy

Employing small molecule inhibitors or activators to precisely regulate key signaling pathways, such as Wnt/β-catenin, YAP/TAZ, SIRT1, NF-κB, and Piezo1. For instance, inhibition of the Wnt pathway can attenuate aberrant cell proliferation and excessive matrix protein accumulation; activation of SIRT1 can safeguard cells against oxidative stress-induced damage and decelerate degenerative processes. Additionally, the development of antibodies or biosimilars targeting specific inflammatory factors or signaling proteins (e.g., antibodies against pivotal factors in the NF-κB pathway) holds promise for mitigating inflammation and cellular injury.

6.2. Gene therapy

Utilizing gene editing technologies, such as CRISPR/Cas9, to rectify genetic defects underlying degenerative conditions or to augment the expression of protective genes. For instance, enhancing Piezo1 expression to enhance cellular responsiveness towards mechanical signals or modulating YAP/TAZ activity for optimizing cell-matrix interactions; employing viral or non-viral vectors for direct delivery of beneficial genes into disc or CEP cells in order to activate or inhibit specific biological processes.

6.3. Mechanical modulation therapy

Involves the application of physical therapy techniques and customized mechanical devices to regulate the mechanical

environment of IVD, thereby optimizing the distribution of compressive and tensile stresses while reducing shear stress-induced cellular damage. Additionally, specialized spinal supports or braces are utilized to alleviate load on damaged discs, promoting natural healing processes by improving posture and alleviating pressure.

The ultimate objective of these therapeutic strategies is to comprehensively regulate the degenerative milieu through diverse modalities, thereby achieving more efficacious disease management and enhancing patient quality of life. As further investigation into these signaling pathways and cellular responses progresses, future interventions will become increasingly personalized, directly targeting the underlying causes of degenerative disorders.

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Data availability statement

Data included in article/supp. material/referenced in article.

Ethics statement

This study was approved by the Management Committee of the Medical School of Suzhou University and the Ethics Committee of Suzhou University (Approval No.: SUDA20230911A01).

CRediT authorship contribution statement

Pan Xiang: Writing – original draft. **Zong-Ping Luo:** Resources, Funding acquisition. **Yan-Jun Che:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

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Glossary

NP	Nucleus Pulposus
NF-κB	Nuclear Factor Kappa Beta
IVDD	Intervertebral Disc Degeneration
IVD	Intervertebral Disc
EP	Endlate
CEP	Cartilaginous Endplate
BEP	Bony Endplate
ECM	Extracellular Matrix
MiRNA	microRNA
ICMT	Intermittent Cyclic Mechanical Tension
YAP	Yes-associated protein
TAZ	Transcriptional Co-activator with PDZ-binding Motif
SIRT1	Silent Information Regulator Sirtuin 1
NF-kB	Nuclear Factor NF-kappaB
GSK3	Glycogen Synthase Kinase 3
TCF	T-Cell Factor
LEF	Lymphoid Enhancer Factor
OSM	Oncostatin M
ROS	Reactive Oxygen Species

CGA Chlorogenic acid
NLRP3 NACHT, LRR, and PYD domains-containing protein 3
PAM Polyacrylamide

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