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Regulated cell death: Implications for intervertebral disc degeneration and therapy

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ABSTRACT

As a controllable biological process, regulated cell death (RCD) extensively participates in cellular homeostasis, organismal development, and the pathogenesis of diseases. This review addresses the research gaps by synthesising the findings on the complexity of RCD modes and their role in disc degeneration, and summarises the preclinical strategies to alleviate disc degeneration and promote disc repair by regulating RCD.

Background: Intervertebral disc degeneration (IDD) is the major source of chronic low back pain. As a controllable biological process, regulated cell death (RCD) extensively participates in the pathogenesis of IDD. Nevertheless, the initiation and progression of RCD remain unclear, and more importantly, the interaction between different RCD modes during IDD and therapy is far from well understood.

Methods: Literature search was performed using "regulated cell death AND intervertebral disc degeneration" in PubMed, Embase, and Web of Science. Meanwhile, relevant findings have been reviewed and quoted.

Results: In this review, we discuss the inducing factors of IDD, various modes of RCD in intervertebral disc, the interactions between different RCD modes, as well as the obstacles to achieve disc regeneration. Meanwhile, the research gaps and perspective in studies that targeting RCD are also presented.

Conclusion: Increasing evidence demonstrated the presence of different RCD modes in intervertebral disc during the progression of IDD. RCD in the resident disc cells is probably induced by the multiple factors such as abnormal mechanical loading, nutritional imbalance, inflammation microenvironment, circadian rhythm changes, withdraw of hormones, and other biomechanical factors. A better understanding of the fundamental mechanisms and the interactions between different RCD modes might contribute to the rescuing of disc degeneration and development of promising therapeutics.

Translational potential statement: The Translational potential of this article. This review aims to demonstrate a better understanding of the fundamental mechanisms governing RCD, which might contribute to the rescuing of disc degeneration and to the development of promising therapeutics in a clinical setting.

1. Introduction

Worldwide, approximately 37% of the adult population suffers from low back pain, which places a huge economic burden on the patients and society, especially with the aging of population [1]. Intervertebral disc degeneration (IDD) is regarded as the major source of chronic low back pain [2]. The intervertebral disc (IVD), a soft tissue connecting the vertebral bodies of the spine, consists of nucleus pulposus (NP), annulus fibrosus (AF) and cartilaginous endplates (CEP). In the degenerated IVD, the function of disc cells is impaired and their number declines, accompanied by the degradation of the extracellular matrix [3]. As a critical subclass of cell death, regulated cell death (RCD) involves strict signal transduction and molecular mechanisms [4] and participates in the degenerative alteration of disc tissues [5]. For instance, RCD in NP cells is

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associated with the loss of disc height and RCD in the AF cells may accelerate the progress of disc herniation [6–8]. In addition, healthy CEP plays an important role in regulating the movement of nutrients [9]. Excessive CEP cells apoptosis is one of the major processes during CEP degeneration [10], which hinders the nutrient supply of disc [11]. Therefore, IDD was inextricably bound to features of the death of IVD cells.

Several RCD modes, such as apoptosis, autophagy-dependent cell death, necroptosis, ferroptosis, pyroptosis and lysosome-dependent cell death, have been reported in IDD. Nevertheless, the initiation and progression of RCD in IVD remain unclear. Moreover, the interaction between different RCD modes during disc degeneration and repair is underexplored. In this review, we synthesise evidence on how physical and pathological environments cause RCD in the IVD, and summarise the RCD modes and their roles in disc degeneration and how different RCD modes interact to initiate disc degeneration. We also discuss the exisiting therapeutic strategies that target RCD for the degenerated disc diseases. A synthesis of studies on RCD could shed light on the mechanisms of disc degeneration and the development of potential therapeutic strategies for degenerative disc diseases.

2. What causes RCD in IVD?

The biomechanical role of IVD is caused by its unique anatomy. Abnormal alterations in the mechanical loading, nutrition, physical and biochemical environments and many other stimuli can cause RCD in IVD cells.

2.1. Mechanical load

The complex structure of IVD allows it to absorb and disperse mechanical loads from physical activities [12]. NP maintains certain hydrostatic pressure required to resist compression; AF is tensioned by radial pressure from the NP and stretch from the CEP [13]. Various modes of mechanical load lead to RCD in IVD cells. Static axial compressive load at 1.3 MPa on rat tail for 1 day or 7 days led to increased apoptotic rate in both NP and AF cells [14]. Additionally, rat NP cells were induced to undergo apoptosis under the action of cyclic stretching at 0.5 Hz and 20% elongation for 24–48 h [15]. These excessive compressions caused apoptosis of IVD cells [16] and promoted the progress of IDD [17]. Collectively, these studies suggest the physiological relevance between IDD and excessive mechanical load, such as overweight, inappropriate sitting posture and heavy manual labor. The importance of mechanical load should be explored in further IDD research.

2.2. Nutrient starvation

During disc degeneration, the source of nutrient supply reduces sharply due to the impaired endplate pathway. Degeneration-related CEP changes hinder the diffusion of metabolites [11]. Atherosclerosis of vertebral vessels is another risk factor that restrict the transport of nutrient into IVD [9]. The viable distance from the nutrient supply reduced with an increase in cell density in IVD. This leads to the lowest amount of glucose concentration and oxygen content in the central NP tissue. Therefore, NP cells are among the first population that undergo nutrient starvation related cell death [18]. Nutrient deprivation by culturing NP cells with 1% FBS for 24 h would induce apoptosis [19]. Enhancing the retolerance to nutrient starvation in NP cells may resistant RCD and become a new therapy for IDD.

2.3. Low pH

Matrix acidity is a major characteristic of the IVD microenvironment; the pH value is between 7.0 and 7.2 in a normal disc and ranges from 6.8 to 6.2 in the degenerated one [20]. Although IVD cells can naturally adapt to this acidic environment, the deteriorated environment imposes challenges on the survival of IVD cells. Previous studies showed that the proteoglycan synthesis of human NP peaked at pH 7.2–6.9 and dropped sharply when the pH was lower than 6.8; the proteoglycan synthesis was only 20% of the normal level at pH 6.3 [21]. In rat NP cells, the lactate elevated after degeneration and in turn enhanced the autophagic level and apoptosis of NP cells [22]. Additionally, *in vitro*, lactate at 6 mM could promote pyroptosis in human NP cells [23].

2.4. Abnormal metabolic condition

Obesity and type 2 diabetes mellitus increase the proportion and severity of disc herniation [24]. The two types of metabolic syndrome pathological conditions are often accompanied by hyperglycemia, an elevated level of accelerated synthesis of advance glycation end products (AGEs), and fat accumulation. High glucose and AGEs directly promote RCD of IVD cells [25]. High glucose (0.1 and 0.2 M) induced the apoptosis of rat CEP cells by inducing excessive reactive oxygen species (ROS) [26]. Additionally, *in vitro*, AGEs at 50 μg/m could significantly promote the apoptosis of rabbit AF cells [27]. Excessive apoptosis of NP cells induced by AGEs may aggravate the progression of IDD [28].

Obesity has also been recongnized as a pro-inflammation physiological condition that destroys the IVD microenvironment. Adipokines, which are metabolism regulators produced by adipose tissue as well as cartilage, contribute to the chronic low-grade inflammation and augment apoptosis in chondrocytes [29,30]. For example, leptin and visfatin were upregulated in degenerated IVD tissues and could contribute to the apoptosis of IVD cells [31,32]. The previous studies highlight the role of pathological metabolism in IDD [33]. However, the pathogenesis process remains largely unknown and requires further research.

2.5. Circadian rhythms

Emerging evidence indicates that a disruption to the circadian rhythm could accelerate tissue degeneration [34]. Shift work, which led to a disordered circadian rhythm, was found to correlate with low back pain [35]. Recently, Bmal1 has been found to be the core circadian rhythm gene of IVD, and the depletion of Bmal1 could lead to disc degeneration in mice [36]. The evidence indicates that dysfunction of the circadian rhythm may be an important inducer of IDD. In cartilage, the deletion of clock gene Bmal1 could also lead to the apoptosis of chondrocytes [37]. Nevertheless, whether abnormal circadian rhythms directly drive RCD in the degenerated IVD remains unclear.

2.6. Withdraw of hormones

For the past decades, increasing attention has been paid to the effect of hormones on degeneration diseases [38,39]. The withdraw of oestrogen can drive programmed cell death in postmenopausal women and leads to higher prevalence of IDD than men [40]. Oestrogen can decrease the apoptosis of IVD cells by inhibiting inflammatory factors, upregulating the PI3K/Akt pathway, and promoting autophagy [41]. A supplement of oestrogen receptors might also aggravate the degenerative changes in IVD cells [42]. These findings suggest the role of oestrogen in inhibiting RCD in IVD cells. Although the impact of oestrogen on IDD in female has been recognized, the role of androgen deficiency in disc degeneration has not been well understood. Androgen deficiency in the aging male has been found to correlated with various musculoskeletal diseases [43]. Normal testosterone level maintained chondrogenic extracellular matrix synthesis in male IVD cells [44]. The results indicate the necessity of investigating the role of androgen deficiency in RCD of IVD cells in future research.

2.7. Other biochemical factors

Other biochemical factors also induce the RCD of IVD cells. Several

local anaesthetics used in the treatment of spine-related pain presented cytotoxicity on IVD cells, such as bupivacaine lidocaine and ropivacaine [45]. A more recent study found that anaesthetics induced apoptosis in IVD cells [46]. Also, bacterial infections such as propionibacterium acnes could induce IDD by promoting the apoptosis and pyroptosis of NP cells [47,48]. Chronic pain caused by disc degeneration can stimulate the releasing of stress hormone cortisol. Cortisol exposing at a physiological level (150 ng/ml) enhanced the pathological cellular processes and programmed cell death in IVD cells [49,50]. In addition, inflammation and N6-methyladenosine (m6A) modification also promoted the progression of IDD [51–53].

Of note, the aforementioned factors do not induce the RCD of disc cells alone; they work together in affecting IVD cells. For example, alterations to CEP cells might impair the delivery of essential nutrients into disc, leading to nutrient starvation in NP. Nutrient deprivation aggravates the low PH environment in IVD, which in turn results in RCD of IVD cells. Thus, it is necessary to draw the atlas of complex microenvironment in IVD.

3. RCD modes and their roles in disc degeneration

IVD cells undergo excessive RCD during disc degeneration, which is the major cause of the decrease in cellular number and viability. Fig. 1 lists the main modes of RCD in IDD.

3.1. Apoptosis in IDD

Apoptosis is a procedural, self-destruction process which eliminates

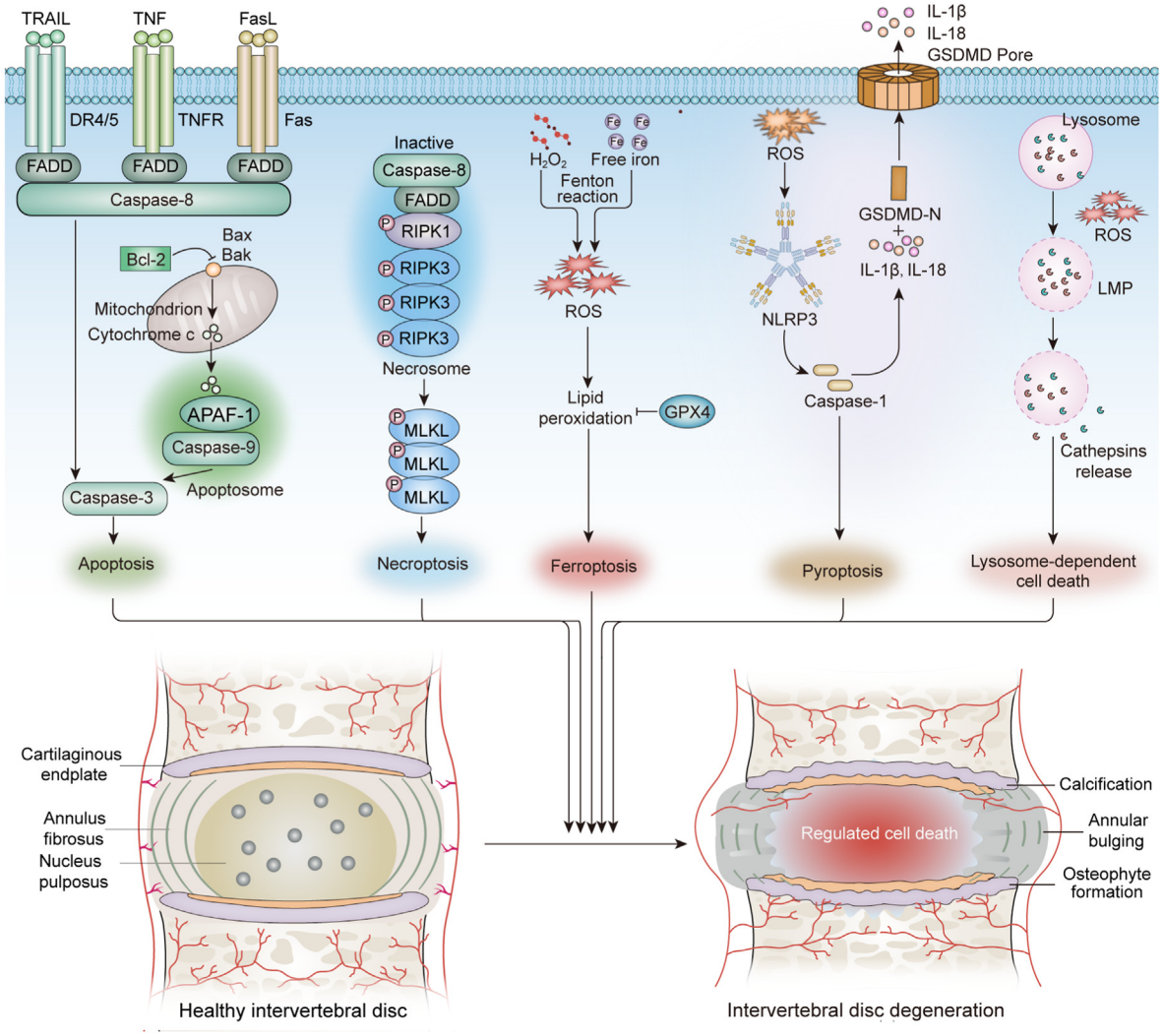

Fig. 1. The occurrence of RCD in IDD. Intervertebral disc (IVD) cells undergo regulated cell death (RCD) under the special biochemical environment and certain stimuli, and final resulting in IDD. Apoptotic pathway can be induced by both the extrinsic death receptor pathway and intrinsic mitochondrial pathway. For intrinsic apoptosis, intracellular stress inhibits Bcl-2 and actives Bcl-associated X protein (Bax) to form pores in the mitochondrial outer membrane; then the cytochrome c is released from mitochondria to cytosol, where it binds to the adaptor protein apoptotic protease activating factor 1 (APAF-1) and caspase 9, leading to the formation of the apoptosome. This event then results in cleavage of caspase-3 and induces apoptosis. Inhibition of caspase-8 enables the formation of the necrosome, and subsequent the phosphorylation and activation of mixed lineage kinase domain-like protein (MLKL), which results in the perforation of cell membrane and necroptosis. Accumulated free iron in IVD cells will react with H_2O_2 through Fenton reaction to drive lipid peroxidation, which can be detoxified and neutralized by glutathione peroxidase 4 (GPX4); excessive lipid peroxidation will lead to ferroptosis. Stimuli also trigger the formation of NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome complex and activate caspase-1, which then promotes the release of IL-1β and IL-18 though gasdermin D (GSDMD) pores. Reactive oxygen species (ROS) can induce lysosomal membrane permeabilization (LMP) to cause cathepsins release, thus triggering lysosome-dependent cell death. Abbreviations: ATG, autophagy-related; DR4, death receptor 4; DR, death receptor; FADD, Fas-related death domain; Fasl, Fas ligand; GSDMD-N, N-terminal fragment of GSDMD; RIPK, receptor-interacting protein kinase; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRAIL, TNF-related apoptosis-inducing ligand; ULK1, Unc-51-like kinase 1.

redundant and unnecessary cells and protects cells from major genotoxic damage. The signal transduction pathways of apoptosis in IVD mainly include the death receptor pathway, the mitochondrial pathway, and the endoplasmic reticulum (ER) pathway [5].

Researchers found that Fas and Fas ligand (FasL) levels were significantly high in IVD tissues from patients with lumbar IVD herniation [54]. In addition, death receptor 4 (DR4) was strongly expressed in degenerated herniated discs, but weakly expressed in normal discs [55]. In the presence of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), DR4 and DR5 were related to the apoptosis of IVD cells and IDD [56]. These studies suggest that death receptor-mediated apoptosis of IVD cells counts for IDD. After treatment using local anaesthetics and AGEs, the mitochondrial pathway resulted in disc cell death [27,46]. In cyclic stretch-induced AF cell apoptosis, the level of ER stress markers, including C/EBP homologous protein and glucose-regulated protein 78, increased obviously [15].

Studies have demonstrated that apoptosis in IVD can be mediated simultaneously by two or three of the aforementioned pathways. For instance, the mitochondrial pathway and death receptor pathway could collaboratively participate in the post-traumatic IDD [57]. In the notochordal cell apoptosis induced by the rat tail static compression, the death receptor pathway could induce transient apoptotic activation, while the p53-mediated mitochondrial pathway promoted continuous apoptosis activation [58]. Importantly, these apoptotic signaling pathways might work at the different stages of IDD. The ER pathway was the main cause of mild degeneration, in which glucose-regulated protein 78 and GADD153 were up-regulated. The death receptor pathway dominated the mild and moderate degeneration, as the activation of caspase-8 were noted. The mitochondrial pathway mainly worked at the moderate and severe stages because the cytochrome c accumulated and caspase-9 was activated [59].

As shown in Supplementary Table 1, several protective factors were employed to prevent IVD cells from apoptosis. Most of these factors, which were examined by *in vitro* studies, targeted at one apoptotic pathway. As the apoptosis pathways might work at the different stages, investigation that determine whether those protective factors can synergistically treat cellular apoptosis through targeting various stages of IDD would be of significance.

3.2. Necroptosis in IDD

Necroptosis is a mixed lineage kinase domain like (MLKL)-mediated necrosis, which is characterized by permeabilization of the plasma membrane, cell rupturing and releasing intracellular components. The intracellular components then cause inflammation [60].

The regulation process of necroptosis is precise and complex and is closely related to apoptosis (Fig. 2). Necroptosis in the NP-derived stem cells under compression was found to impede the endogenous repair of IVD [61]. In the IVD, necroptosis and apoptosis are closely linked; the apoptotic pathway might partially develop towards necroptosis if the apoptosis of NP cells was inhibited [62]. This postulation points to the complexity of the cross-talk among RCD modes as listed in Fig. 2. Biologic drugs that inhibit the key molecules involved in necroptosis as well as apoptosis should be trialed in future studies of IDD.

3.3. Ferroptosis in IDD

Ferroptosis is initiated by iron-dependent phospholipid peroxidation, characterised by the accumulation of lipid hydroperoxides to lethal levels and regulated by a variety of cellular metabolic pathways [63]. Recently, studies demonstrated that ferroptosis pathway were enriched in subgroups of degenerated IVD cells using single-cell RNA-sequencing [64]. The ferroptosis of NP cells can be induced by homocysteine through glutathione peroxidase 4 (GPX4) methylation [65]. In addition, ferroportin dysregulation caused intercellular iron overload and subsequently led to ferroptosis in IVD cells [66]. The preliminary findings suggest that ferroptosis can serve as a novel target for IDD therapy, despite those extensive experiments are required before clinical application.

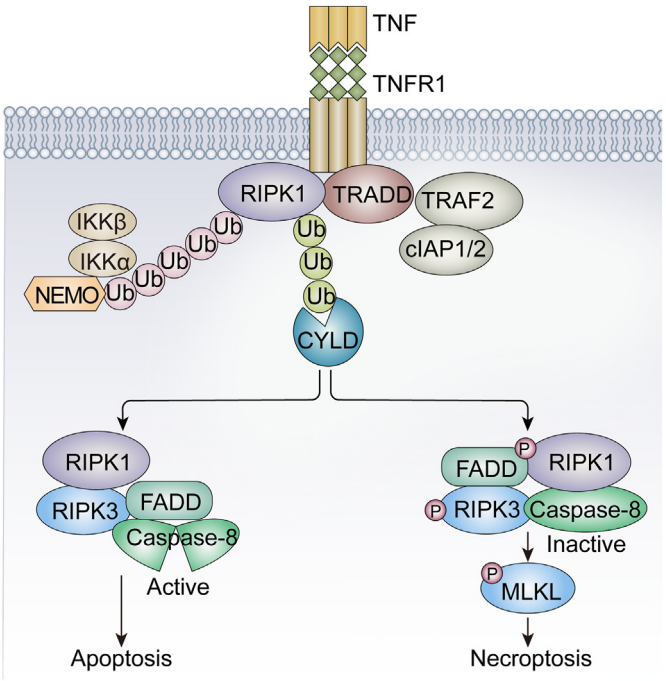

Fig. 2. Death receptor is involved in the necroptosis signal pathway and is closely related to apoptosis. The death receptor pathway is activated when tumor necrosis factor (TNF) binds to TNF receptor 1 (TNFR1). Subsequently, TNFR1-associated death domain protein (TRADD) and receptor-interacting protein kinase 1 (RIPK1) are recruited to the intracellular death domain to form complex. TRADD recruits the inhibitor of apoptosis 1 (IAP1) and IAP2 to the complex, and then the complex is deubiquitylated by cylindromatosis (CYLD). Upon the inhibition of caspase 8, active RIPK3 phosphorylates the mixed lineage kinase domain like (MLKL), thereby causing conformational changes of MLKL. Then MLKL translocates to the plasma membrane, leading to necroptotic cell death. By contrast, the activated caspase 8 pre-empts the activation of necroptosis and results in apoptosis. Abbreviations: FADD, Fas-related death domain; IKK, IκB kinase; MLKL, mixed lineage kinase domain-like protein; NEMO, NF-κB essential modulator; TRAF2, TNFR-associated factor 2; Ub, ubiquitination.

3.4. Pyroptosis in IDD

Pyroptosis is another form of lytic cell death that is mediated by the cleavage and activation of members of the gasdermin (GSDM) superfamily, mainly gasdermin D (GSDMD). Upon the formation of GSDMD pores, pro-inflammatory factors are released, the cells then undergo swelling and the plasma membrane ruptures [67].

The pyroptosis of NP cells was found to be induced by acid-sensitive ion channel 1a (ASIC1a) and ASIC3 under the stimulation of lactate, which further promoted IDD [23]. Notably, autophagy could inhibit LPS-induced NP cell pyroptosis, thus delaying the progression of IDD [68].

3.5. Lysosome-dependent cell death in IDD

Lysosome-dependent cell death is a type of RCD which is characterised by lysosomal membrane permeabilization (LMP). The lysosomes tend to leak when cells are exposed to dipeptide methyl esters, lipid metabolites and ROS [4]. Bupivacaine can induce lysosome-dependent cell death in both NP and AF cells. With the concentration of bupivacaine increasing, the size of the lysosomes in the IVD cells grew and then disintegrated, leading to LMP with the release of cathepsins.

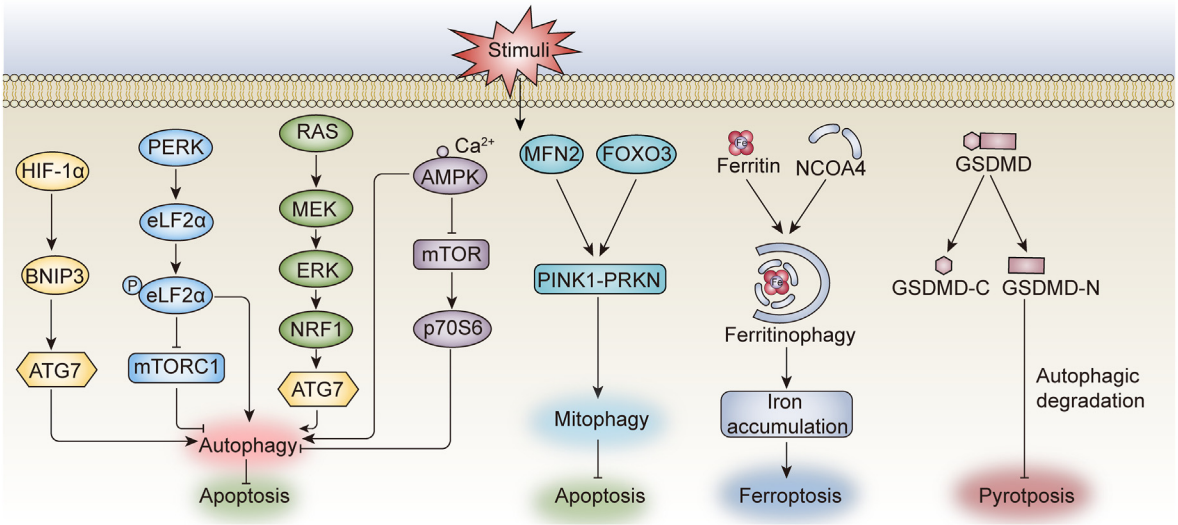

Fig. 3. The interaction between RCD modes in IDD. In IDD, multiple RCD modes usually exist simultaneously, and they interact closely with each other. Activation of the autophagy can effectively inhibit apoptosis in the induced disc degeneration models. Additionally, Activation of the mitophagy through phosphatase and tensin homolog (PTEN)-induced putative kinase 1 and parkin RBR E3 ubiquitin protein ligase (PINK1/PRKN) pathway also suppressed the apoptosis of IVD cells. Ferritinophagy, a nuclear receptor coactivator 4 (NCOA4)-mediated ferritin selective autophagy, mediates the ferroptosis of IVD cells. Furthermore, autophagy was found to protect cells from pyroptosis via the degradation of N-terminal fragment of gasdermin D (GSDMD-N). Abbreviations: AMPK, AMP-activated protein kinase; ATG, autophagy-related; eIF2α, eukaryotic translation initiation factor 2α; FOXO3, forkhead box O3; GSDMD-C, C-terminal fragment of gasdermin D; HIF, hypoxia inducible factor; MFN2, mitofusin 2; mTOR, mammalian target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; PERK, protein-kinase-R-like endoplasmic reticulum kinase.

Furthermore, ROS is the key mediator of the above process. This evidence demonstrates that bupivacaine-induced IVD cell death is related to LMP and lysosomal cathepsins releasing [69].

Importantly, LMP is capable of triggering apoptosis, pyroptosis, ferroptosis and necrosis, depending on the cell type and the extent of lysosomal membrane damage [69,70]. Partial and selective LMP might lead to apoptosis and inflammasome-dependent pyroptosis, while complete lysosomal rupture caused necrosis [70]. Additionally, LMP may amplify in the context of apoptosis, autophagy-dependent cell death and ferroptosis [4]. These findings suggest a core role of lysosome-dependent cell death in the RCD network. Controlling lysosomal leakage should thus be used as a potential strategy to protect cells from RCD in disc degeneration diseases (Fig. 3).

4. Interaction between autophagy and RCD in IDD

Many studies have examined the interaction between autophagy and other RCD modes in IDD. Autophagy is a process for cells to adapt to environmental changes and maintain homeostasis; if autophagy is not active enough, defective organelles and proteins will accumulate, resulting in apoptosis. Autophagy activation can effectively inhibit apoptosis in IDD [71]. Restoration of the autophagic flux has been shown to inhibit the apoptosis of NP cells and retard the development of IDD [72]. Through the HIF-1α-BNIP3-ATG7 axis-mediated autophagic flux, NP-derived stem cells with overexpressed HIF-1α exhibited stronger resistance to over-loading induced apoptosis [71]. Another effective way to reduce apoptosis was by activating mitophagy to eliminate damaged mitochondria. In a punctured IDD model, mitophagy scavenged the dysfunctional mitochondria to inhibit ROS production and the apoptosis of NP cells [73]. Additionally, autophagy was found to protect human NP cells from pyroptosis via a P62/SQSTM1-mediated degradation of GSDMD-N [68]. By contrast, cell death wound occurred if autophagy destroyed the organelles excessively [74]. Autophagy also participated in ferroptosis in IDD, as ferroptosis was found to be an autophagic degradation dependent cell death [75]. Furthermore, the inhibition of ferritinophagy, a NCOA4-mediated ferritin selective autophagy, can protect cells from TBHP-induced ferroptosis [76].

5. Therapeutic strategies that target RCD for degenerated disc diseases

Strategies for the therapy of disc degeneration are based on the premise that degeneration partly results from the RCD, which can be reversed or rescued accordingly. Some of these strategies have been shown to be successful in preclinical.

5.1. Exogenous stem cell-based approaches

The aim of cell-based approaches is to rapidly replenish the damaged cells or reverse the pathologic RCD process in IVD. Exogenous mesenchymal stem cells (MSCs) have been reported to alleviate the apoptosis of NP cells through enhancing autophagy [77]. Despite the success of cell-based therapy for IDD, several challenges remain. Transplanted stem cells are unable to survive in the hypoxia and low pH environment of IDD [3]. Additionally, an insufficient number of transplanted MSCs might not be enough to reverse the RCD process of disc cells, but the surplus exogenous cells might compete for nutrients in the avascular environment and lead to severe cell death in the degenerated IVD [9,78]. Therefore, the number of exogenous cells should meet the need for repairing while avoiding fierce competition for an already unstable supply of nutrients.

5.2. Endogenous progenitor-based approaches

Despite the notable success, the clinical application of exogenous stem cells has some limitations. Recently, endogenous progenitor cells have been identified in different compartments of IVD, suggesting the self-repair potential of IVD. There is evidence that endogenous progenitor cells can release exosomes to inhibit the apoptosis of NP cells [79]. Additionally, IVD progenitors may have huge potential in IVD cell differentiation as they could adapt to the microenvironment. However, the microenvironment of degenerative IVD may affect the survival of IVD progenitor cells and cause them to undergo RCD in the process of IDD [61]. In degenerative IVD, the hostile microenvironment reduces the migration, proliferation and stemness of IVD progenitors [9]; the

persistent adverse factors are likely to lead to the RCD of progenitors and, consequently, the failure of endogenous repair [80]. At this point, maintaining the intrinsic repair potential of progenitor cells and enhancing the recruitment of endogenous repair cells are promising strategies to retard IDD [71].

5.3. Exosome-based approaches

The rationale for cell-based approaches discussed above was the replenishing of dead IVD cells by differentiation of grafted MSCs. However, overwhelming evidence from preclinical studies demonstrated that the tissue repair effects of stem cells were driven by exosomes released from functional cells [81]. Therefore, exosomes can result in similar effects of stem cell therapy while avoid side effects of cell-based approaches [82,83].

The exosomes obtained from MSCs and IVD progenitors have been widely used in the treatment of IDD. For instance, the exosomes derived from MSCs could govern the fate of NP cells by attenuating apoptosis and pyroptosis [28,84], and activating autophagy [79]. The therapeutic effect of exosomes was determined by the cargo, such as miRNAs [81]. Elucidating the cargoes contained in exosomes and their function helps to make better use of exosomes for IDD. Furthermore, the use of exosomes for drug delivery is also promising. Nevertheless, how to manufacture sufficient exosomes efficiently for IDD therapy remains a significant challenge for both researchers and physicians.

5.4. Non-coding RNA-based approaches

Table 1 displays the important role of non-coding RNAs in the regulation of RCD in the IVD; the microRNAs (miRNAs), circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) are mainly employed in the regulation of RCD in IVD cells [85,86].

Different miRNA expression profiles have been observed in patients with IDD. Anomalously expressed miRNAs, such as miR-141 and miR-199a-5p, were found to induce the apoptosis of NP cells and accelerate the process of IDD [87,88]. On the other hand, miRNAs could inhibit the occurrence of RCD in IDD. For instance, the overexpression of miR-140–3p retarded IDD [89]. Additionally, the expression of miR-27a could be triggered by the degenerative microenvironment to inhibit apoptosis of IVD cells [90]. CircRNAs perform biological functions by acting as inhibitors of miRNAs or proteins; they regulate RCD mainly through a competing endogenous RNAs (ceRNA) mechanism. The down-regulation of circ-GRB10, circVMA21 and circRNA-CIDN was observed in the degenerated IVD. These circRNAs prevented IDD by inhibiting RCD-related genes in a ceRNA way [91–93]. LncRNA ANPODRT could protect NP cells from apoptosis by activating Nrf2 signal transduction [94]. Further, LncRNA HOTAIR promoted apoptosis through stimulating the autophagy of NP cells, making silencing HOTAIR a potential treatment for IDD [95]. Under nutritional stress, LINC00641 could regulate autophagy and suppress IDD by targeting miR-153–3p and ATG5 [96].

Non-coding RNAs have exhibited great potential to function as key regulators in reversing RCD in degenerated IVD, but challenges still remain. First, non-coding RNAs participate in cellular activity through a complex gene regulatory network rather than acting as molecular switches in a separate pathway; some miRNAs may inhibit apoptotic genes while activating certain RCD pathways [97]. Second, how to regulate safely these non-coding RNAs *in vivo* remains challenging. Transfection of circRNA-producing plasmid can be used to overexpress circRNAs in cell lineages or animal models, but this procedure might result in off-target or interfering with other genes. Moreover, developing drugs that target non-coding RNAs is a promising exploitation, but its implication in IDD still requires further research [97]. Taken together, the functions of non-coding RNAs enable them to be novel targets in RCD of IVD cells, but challenges remain in the application of these approaches in clinical treatment.

5.5. Cytokine-based approaches

Growth factors, such as transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs), are essential in maintaining IVD homeostasis [98,99]. They also possess the potential to alleviate RCD in discs. For instance, recombinant human BMP2/7 could block apoptosis in NP cells and reduce the occurrence of IDD in aging rats [100]. Despite the lack of *in vivo* evidence, previous studies found that additional TGF-β protected NP cells against apoptosis [101]. The hepatocyte growth factor and fibroblast growth factor 2/18 also exhibited anti-apoptotic function in IVD cells [102–104]. The findings reveal that cytokines can be used to promote the proliferation and prevent apoptosis of IVD cells, thus inhibiting the degenerative process. Nevertheless, excessive growth factors can be detrimental to IVD. For example, aberrant activation of TGF was found to contribute to the abnormal cell fate changes of AF [105]. Furthermore, the repair of IVD is a slow and continuous process that requires persistent administration of the growth factors. Thus, a controllable drug delivery system is necessary for facilitating the application of cytokine-based approaches.

5.6. Biomaterial approaches

At present, the primary aims of biomaterials for IDD are either as a container for cell encapsulation to replenish the damaged cells or as a biocompatible scaffold to restore disc height and improve disc biomechanics [106]. Biomaterials can act as a safe house to protect the implanted cells from apoptosis [107]. Furthermore, functionalised biomaterial can serve as a carrier and controlling release system for the inhibitors of inflammatory cytokines or degenerative factors such as IL-1 receptor antagonist [108,109], thus improving the harsh microenvironment and avoiding RCD of both the exogenous and endogenous cells [110]. For example, ferulic acid-incorporated thermosensitive chitosan/gelatin/glycerol phosphate hydrogel inhibited apoptosis of H_2O_2-induced NP cells [111]. Through the coupling of APETx2 and loading NP cells, the injectable hydrogel microsphere modulated the

Table 1
Effects of different non-coding RNAs on the RCD in IDD.

Non-coding RNAs		Expression in IDD	Target	Effect on RCD	References
MiRNA	miR-141	↑	SIRT1/NF-κB	Promoting apoptosis	[87]
	miR-199a-5p	↑	SIRT1, p21	Promoting apoptosis	[88]
	miR-140–3p	↓	KLF5/N-cadherin/MDM2/Slug	Inhibiting apoptosis	[89]
	miR27a	↓	FSTL1	Inhibiting apoptosis	[90]
CircRNA	circ-GRB10	↓	miR-141–3p, FUS	Inhibiting apoptosis	[91]
	circVMA21	↓	miR-200C, XIAP	Inhibiting apoptosis	[92]
	circRNA-CIDN	↓	miR-34a-5p, SIRT1	Inhibiting apoptosis	[93]
LncRNA	ANPODRT	↓	Keap1-Nrf2	Inhibiting apoptosis	[94]
	HOTAIR	↑	AMPK/mTOR/ULK1 pathway	Promoting apoptosis and autophagy	[95]

Abbreviations: AMPK, AMP-activated protein kinase; FSTL1, Follistatin-like protein 1; Keap1, kelch-like ECH-associated protein 1; KLF5, Krüppel-like Factor 5; mTOR, mammalian target of rapamycin; SIRT1, silent information regulator 1; ULK1, Unc-51-like kinase 1.

Table 2
Compounds alleviating IDD by inhibiting RCD *in vivo*.

Compounds	Mechanisms of RCD inhibition	Pharmacological effects	Species	References
Metformin	AMPK	Stimulating autophagy in NP cells, which confers an anti-apoptosis effect and ameliorates IDD	Rat	[118]
Curcumin	AMPK/mTOR/ULK1	Enhancing autophagy to resist apoptosis and IDD	Rat	[122]
Ligustilide	NF-κB	Inhibiting apoptosis of NP cells and retarding IDD	Rat	[113]
Honokiol	TXNIP/NLRP3/caspase-1/IL-1β, NF-κB/JNK	Inhibiting the H_2O_2 induced apoptosis and suppressing the pathogenesis of IDD	Rat	[114]
Platelet-derived growth factor BB	—	Inhibiting apoptosis and retarding disc degeneration	Rabbit	[124]
Luteoloside	NF-κB	Inhibiting apoptosis and ameliorating IDD	Rat	[117]
Duhuo jisheng decoction	p38/MAPK	Preventing IDD by activating autophagy	Rat	[115]
Kinsenoside	AKT-ERK1/2-Nrf2	Protecting against apoptosis to ameliorate IDD	Rat	[116]
BMP 2	PI3K/Akt	Alleviating IDD by inhibiting NP cells apoptosis	Rat	[125]
Spermidine	Collagen II/Adamts-5	Protecting NP cells against apoptosis through autophagy activation to alleviate IDD	Rat	[119]
Rapamycin	Nrf2/Keap1	Enhancing autophagy to protect CEP from chronic degeneration	Mouse	[120]
Berberine	Bax, Bcl-2	Stimulating autophagy against apoptosis and preventing the development of IDD	Rat	[121]
Urolithin A	AMPK	Inhibiting apoptosis of NP cells by activating mitophagy, thus retarding the progress of IDD	Rat	[126]
Cortistatin	NLRP3, NF-κB	Antagonizing apoptosis in NP cells and alleviating IDD	Mouse	[127]
MCC950	NLRP3	Inhibiting pyroptosis and attenuating IDD	Rabbit	[123]
Folic acid	GPX4	Resisting ferroptosis and alleviating IDD	Mouse	[65]

Akt, protein kinase B; AMPK, AMP-activated protein kinase; Bax, Bcl-2-associated X protein; GPX4, glutathione peroxidase 4; IDD, intervertebral disc degeneration; JNK, c-Jun N-terminal kinase; Keap1, kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NF-κB: nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; RCD, regulated cell death; TXNIP, thioredoxin-interacting protein; ULK1, Unc-51-like kinase 1.

overactive inflammation environment, which was considered the main RCD-inducing factor [112]. Thus, biomaterial-based approaches have become a powerful tool against RCD in IVD.

6. Evidence from preclinical trials

Even though the new methods mentioned above open new possibilities, there is a need to test drugs, which has been proven safe and effective in other degenerative diseases, for the treatment of the IDD. Table 2 lists the studies focusing on compounds that inhibit RCD in IVD and the underlying mechanisms. Among them, ligustilide, honokiol, duhuo jisheng decoction, kinsenoside and luteoloside could resist apoptosis [113–117]; curcumin, metformin, spermidine, rapamycin, and berberine could promote autophagy [118–122]. Additionally, MCC950 inhibited pyroptosis, thus delaying the occurrence of IDD [123]. In all, the pre-clinical application of these "old" drugs in IDD is worth further testing.

7. Future perspectives and conclusions

IDD is closely associated with the RCD of the resident disc cells, which is probably induced by the abnormal alterations of mechanical loading, nutritional balance, microenvironment, circadian rhythm and other biomechanical factors. Nevertheless, the underlying mechanism regarding how these factors induce the occurrence and progress of RCD remains unclear.

The apoptosis and autophagy of disc cells have received increasing attention, while not enough attention has been paid to other RCD modes. Instead of acting as several single links in the cell death process, different RCD modes exist simultaneously and the crosstalk between them seems to occur frequently. Thus, the atlas of the interaction between these modes deserves greater attention. Targeting multiple RCD modes at the same time might be necessary for the development of effective therapies. Unlike the cell-based approaches that involve ethical issues, the non-coding RNAs, exosomes, cytokines and biomaterials, if appropriately designed, can become a new strategy to treat degenerative disc disease through the regulation of RCD. Nonetheless, despite the importance of RCD in disc degeneration, clinical trials mainly emphasise the alleviation of pain and neurological symptoms; little clinical evidence has been obtained to understand how RCD changes after the treatment. To ameliorate the quantity and quality of resident IVD cells, monitoring the RCD using advanced imaging techniques is of great significance.

RCD has already been closely connected with IVD degeneration and therapy. Although the picture of RCD and disc degenerative diseases is becoming increasingly complex, the opportunities to elucidate the complex relationship and to develop advanced therapeutics are also increasing.

Author contributions

All the authors made a substantial contribution in writing, reviewing and editing the manuscript.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

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