Roles of circular RNAs in the pathogenesis of intervertebral disc degeneration (Review)

ZHIDI LIN, FEIZHOU LU, XIAOSHENG MA, XINLEI XIA, FEI ZOU and JIANYUAN JIANG

Department of Orthopedics, Huashan Hospital, Fudan University, Shanghai 200040, P.R. China

Abstract. Lower back pain (LBP) is an extremely common symptom and is recognized as a leading contributor to disability and disease burden globally. Intervertebral disc degeneration (IDD) represents a major cause of LBP. However, the molecular mechanisms involved in the pathogenesis of IDD remain unclear, and currently available treatments, including conservative and surgical options, fail to effectively delay, stop or reverse the progression of IDD. Circular RNAs (circRNAs) are a newly discovered group of covalently closed, single-stranded and endogenous non-coding RNAs. A growing body of research has revealed that a number of circRNAs are widely and aberrantly expressed in IDD tissues. Furthermore, they play important roles in the pathogenesis of IDD, including proliferation, apoptosis, senescence, mitophagy, inflammation and extracellular matrix metabolism, mainly by acting as sponges for microRNAs. The present review aims to summarize the current understanding on the mechanisms of circRNA-mediated regulation in IDD.

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Correspondence to: Dr Fei Zou or Professor Jianyuan Jiang, Department of Orthopedics, Huashan Hospital, Fudan University, 12 Wulumuqi Middle Road, Shanghai 200040, P.R. China
E-mail: zoufei@huashan.org.cn
E-mail: jjy@fudanspine.com

1. Introduction

Lower back pain (LBP) is one of the most common symptoms and/or disorders globally, affecting people of all ages, and is now the number one cause of disability worldwide (1,2). It has been reported that ~84% of the general population suffers from LBP during their lifetime (3), and roughly 10% of them become chronically disabled (4). Due to the extremely high prevalence of LBP, it seriously harms society and the economy, placing a heavy burden on health care systems globally (5).

Intervertebral disc degeneration (IDD) is a major pathological contributor to LBP. The intervertebral disc is a complex fibrocartilaginous tissue that connects adjacent vertebral bodies and confers spinal mobility; it is also the largest avascular structure in the human body, consisting of the nucleus pulposus (NP), annulus fibrosus (AF) and cartilage endplate (CEP). Lacking a blood supply, disc cells retrieve oxygen and nutrients mainly by diffusion through CEPs. Thus, they have a limited capacity for self-repair after damage or degeneration (6). The extracellular matrix (ECM) is mainly synthesized and secreted by NP cells (NPCs) and is composed of type II collagen (collagen II) and aggrecan, helping to resist compression and maintain disc height. According to previous studies, IDD is mainly characterized by dysregulation of NPC survival, an imbalance between ECM anabolism and catabolism, and aberrant activation of the inflammatory response (7,8).

As the molecular mechanisms of IDD are complicated and remain unclear, circular RNAs (circRNAs) have attracted growing attention in the past few years. circRNAs are a novel class of covalently closed, single-stranded and endogenous non-coding RNAs, unlike linear RNAs, without 5'-3' polarities and polyadenylation. Most circRNAs are produced from exons by a backsplicing mechanism in eukaryotes with cell type- and developmental stage-specific expression patterns (9). Compared with linear RNAs, circRNAs are generally more stable and resistant to RNase R digestion owing to their loop structure. circRNAs are abundant and evolutionarily conserved, and mainly localize to the cytoplasm. They exert important biological functions by acting as microRNA (miRNA/miR) sponges, which are also known as competing endogenous RNA (ceRNA) mechanisms, in the pathological process of various diseases (10).

Previous studies have shown that circRNAs play critical roles in various musculoskeletal diseases, including osteoarthritis, rheumatoid arthritis, osteoporosis, osteosarcoma,

osteonecrosis, scoliosis and spinal cord injury (11-17). Recently, accumulating evidence has uncovered the significant differential expression of circRNAs between IDD and control groups. Gain-of-function, loss-of-function and rescue experiments were generally performed to investigate the biological functions and regulatory pathways of circRNAs. Luciferase reporter, pulldown and immunoprecipitation assays were performed to confirm the direct interactions among the molecules of signaling pathways of circRNAs (18,19).

The present review summarizes the current evidence on the roles of circRNAs in the pathogenesis of IDD, including proliferation, apoptosis, senescence, mitophagy, inflammation and ECM metabolism (Table I).

2. Roles of circRNA in ECM metabolism

ECM degradation is a key contributor to the progression of IDD, leading to loss of compression resistance and disc height. Under pathological conditions, the balance between the synthesis and decomposition of the ECM is disturbed, which is characterized by decreased structural components (collagen II and aggrecan) and increased matrix-degrading enzymes [matrix metalloproteinase (MMP)-2, MMP-3, MMP-13, A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs (ADAMTS)-4 and ADAMTS-5] (20-22).

Downregulated expression of circRNAs in ECM metabolism. Cheng *et al* (23) first elucidated the role of a specific circRNA in the pathological process of IDD. circVMA21 was significantly downregulated in IDD tissues compared with control tissues. Overexpression of circVMA21 reversed the imbalance between ECM anabolism and catabolism in NPCs after treatment with tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) *in vitro*. Luciferase reporter, pulldown, RNA immunoprecipitation and fluorescence *in situ* hybridization assays confirmed that circVMA21 directly binds with cytoplasmic miR-200c and subsequently upregulates the expression of the target mRNA, X linked inhibitor-of-apoptosis protein (XIAP).

Wang *et al* (24) found that circSEMA4B overexpression in NPCs attenuated the adverse effects on ECM synthesis induced by IL-1β treatment *in vitro*. It was further verified that circSEMA4B exerted positive functions by sponging miR-431 and subsequently upregulating the expression of secreted frizzled related protein 1 and glycogen synthase kinase-3β, which are known as Wnt signaling-related factors. Similarly, it was reported that circERCC2 facilitated ECM anabolism in NPCs by sponging miR-182-5p and elevating the expression level of silent mating type information regulation 2 homolog 1 (SIRT1) (25).

Guo *et al* (26) suggested that circ-GRB10 enhanced NPC survival through the miR-328-5p-Erb-b2 receptor tyrosine kinase 2 (ERBB2) axis. Notably, another study by Guo *et al* (27) focused on circ-GRB10 to elucidate the upstream mechanisms. Forced expression of circ-GRB10 promoted phosphorylated-Erk1/2 expression and ameliorated ECM synthesis. RNA-binding protein FUS (FUS) showed strongly downregulated expression, while significant upregulation of miR-141-3p expression was observed in IDD samples. The positive effects induced by FUS overexpression could be counteracted by silencing circ-GRB10. In addition,

RNA immunoprecipitation demonstrated the direct interaction between FUS and circ-GRB10. Moreover, combined with further investigations and the results of the previous study (26), Guo *et al* (27) established the signaling circuitry of the circ-GBR10-miR-328-5p-ERBB2-Erk1/2 phosphorylation-miR-141-3p-FUS loop in the pathogenesis of IDD (Fig. 1).

Chen *et al* (28) confirmed that circGLCE attenuates ECM degradation by competitively absorbing miR-587 to upregulate signal transducing adaptor family member 1 expression. Huang *et al* (29) reported that circPKNOX1 facilitated ECM synthesis and restrained ECM decomposition. Dual luciferase assays indicated that circPKNOX1 directly interacts with miR-370-3p and subsequently upregulates KIAA0355 expression.

Upregulated expression of circRNAs in ECM metabolism. Wang *et al* (30) identified the significant upregulation of circ-4099 expression in NPCs after treatment with TNF-α *in vitro*. Overexpression of circ-4099 promoted ECM anabolism, which was rescued by miR-616-5p mimics. Further experiments revealed that circ-4099 ameliorates ECM synthesis through the miR-616-5p-SRY-related high mobility group-box gene 9 (Sox9) axis. It was reported that knockdown of circRNA_104670 increased ECM synthesis, which was counteracted by miR-17-3p inhibition (31). Luciferase reporter and enhanced green fluorescent protein/red fluorescent reporter assays demonstrated that circRNA_104670 aggravates ECM degradation by sponging miR-17-3p and upregulating MMP-2 expression. Cui and Zhang (32) showed that silencing circ_001653 in NPCs ameliorated ECM synthesis and inhibited ECM decomposition. It was further determined that circ_001653 sponges miR-486-3p, which targets cell migration-inducing hyaluronan binding protein (CEMIP) to regulate ECM metabolism.

In the study by Xiang *et al* (33), compression treatment rather than inflammatory cytokines (ICs) was applied to treated NPCs and induced IDD. Highly expressed in IDD samples and compression-treated NPCs, circRNA-CIDN could ameliorate the pro-catabolic effects in NPCs induced by compression *in vitro* and *ex vivo*. Luciferase reporter and RNA immunoprecipitation assays verified the direct binding between circRNA-CIDN and miR-34a-5p, and between miR-34a-5p and SIRT1. Rescue experiments indicated that circRNA-CIDN overexpression blocked the pro-catabolic effects of the miR-34a-5p mimic, while knockdown of SIRT1 impaired the protective effects of circRNA-CIDN in compression-treated NPCs. circRNA-CIDN therefore alleviates ECM catabolism induced by compression through the miR-34a-5p-SIRT1 pathway. Guo *et al* (34,35) reported that both circ-FAM169A and circ-TIMP2 shift ECM homeostasis towards catabolism in NPCs by functioning as ceRNAs through miR-583-β-transducin repeat containing (BTRC) and miR-185-5p-MMP-2 signaling, respectively.

Xiao *et al* (36) focused on endplate chondrocytes instead of NPCs and found that circRNA_0058097 expression was upregulated in endplate chondrocytes after treatment with intermittent cyclic tension *in vitro*. Tension treatment decreased collagen II, aggrecan and Sox9 expression, and changed the cell shape in endplate chondrocytes. Further assays showed that circRNA_0058097 negatively regulated

Table I. Roles of specific circRNAs in IDD.

First author/s, year	circRNA	Samples	Cell type	Treatment	Expression	miRNA	Targets	Functions	(Refs.)
Cheng et al, 2018	circVMA21	IDD vs. thoracolumbar fracture or scoliosis	NPC	TNF-α and IL-1β	↓	miR-200c	XIAP	ECM synthesis ↑, cell apoptosis ↓	(23)
Guo et al, 2018	circ-GRB10	Lumbar IDD vs. traumatic lumbar fracture	NPC	Nutrition deprivation	↓	miR-328-5p	ERBB2	Cell apoptosis ↓	(26)
Wang et al, 2018	circ-4099	IDD vs. vertebral fracture or scoliosis	NPC	TNF-α	↑	miR-616-5p	Sox9	ECM synthesis ↑, inflammation ↓	(30)
Song et al, 2018	circRNA_104670	Cervical spondylotic myelopathy vs. Hirayama disease	NPC	NA	↑	miR-17-3p	MMP-2	ECM synthesis ↓, cell proliferation ↓, cell apoptosis ↑	(31)
Wang et al, 2018	circSEMA4B	Severe vs. mild lumbar IDD	NPC	IL-1β	↓	miR-431	Wnt pathway (SFRP1 and GSK-3β)	ECM synthesis ↑, cell proliferation ↑, cell senescence ↓	(24)
Xie et al, 2019	circERCC2	Cervical spondylotic myelopathy vs. Hirayama disease	NPC	TBHP	↓	miR-182-5p	SIRT1	ECM synthesis ↑, cell apoptosis ↓, cell senescence ↓, cell mitophagy ↑	(25)
Cui and Zhang, 2020	circ_001653	IDD vs. spinal cord injury	NPC	NA	↑	miR-486-3p	CEMIP	ECM synthesis ↓, cell proliferation ↓, cell apoptosis ↑	(32)
Xiang et al, 2020	circRNA-CIDN	IDD vs. idiopathic scoliosis	NPC	Compression	↓	miR-34a-5p	SIRT1	ECM synthesis ↑, cell apoptosis ↓	(33)
Guo et al, 2020	circ-FAM169A	IDD vs. traumatic vertebral fracture	NPC	NA	↑	miR-583	BTRC, NF-κB pathway	ECM synthesis ↓, inflammation ↑	(34)
Guo et al, 2020	circ-TIMP2	Lumbar IDD vs. traumatic lumbar fracture	NPC	TNF-α and IL-1β	↑	miR-185-5p	MMP-2	ECM synthesis ↓	(35)
Xiao et al, 2020	circRNA_0058097	Severe vs. mild IDD	CEP cell	Tension	↑	miR-365a-5p	HDAC4	Cell morphology ↓, ECM synthesis ↓	(36)
Song et al, 2020	circRNA_0000253	Severe vs. mild IDD	NPC	NA	↑	miR-141-5p	SIRT1	ECM synthesis ↓, cell proliferation ↓, cell apoptosis ↑	(37)
Li et al, 2020	circ-FAM169A	Lumbar IDD vs. thoracolumbar fracture or scoliosis	NPC	NA	↑	miR-583	Sox9	NA	(42)

Table I. Continued.

First author/s, year	circRNA	Samples	Cell type	Treatment	Expression	miRNA	Targets	Functions	(Refs.)
Guo *et al*, 2020	circ-GRB10	Lumbar IDD vs. traumatic lumbar fracture	NPC	NA	↓	miR-328-5p	Erk1/2, miR-141-3p, FUS (upstream targets)	ECM synthesis ↑	(27)
Chen *et al*, 2020	circGLCE	IDD vs. traumatic lumbar fracture	NPC	IL-1β	↓	miR-587	STAP1	ECM synthesis ↑, cell apoptosis ↓	(28)
Kong *et al*, 2020	circ_0059955	IDD vs. normal discs from cadavers	NPC	NA	↓	NA	ITCH	Cell proliferation ↑, cell apoptosis ↓, cell cycle arrest ↓, inflammation ↓	(44)
Huang *et al*, 2021	circPKNOX1	IDD vs. lumbar vertebrae trauma	NPC	NA	↓	miR-370-3p	KIAA0355	ECM synthesis ↑	(29)

circRNA, circular RNA; miRNA, microRNA; IDD, intervertebral disc degeneration; NPC, nucleus pulposus cell; ECM, extracellular matrix; CEP, cartilage endplate; NA, not available; ↑, upregulation; ↓, downregulation; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; XIAP, X linked inhibitor-of-apoptosis protein; ERBB2, Erb-b2 receptor tyrosine kinase 2; Sox9, SRY-related high mobility group-box gene 9; MMP, matrix metalloproteinase; SFRP1, secreted frizzled related protein 1; GSK-3β, glycogen synthase kinase-3β; SIRT1, silent mating type information regulation 2 homolog 1; CEMIP, cell migration-inducing hyaluronan binding protein; TBHP, tert-butyl hydroperoxide; BTRC, β-transducin repeat containing; NF-κB, nuclear factor-κB; HDAC4, histone deacetylase 4; FUS, RNA-binding protein FUS; STAP1, signal transducing adaptor family member 1; ITCH, itchy E3 ubiquitin protein ligase.
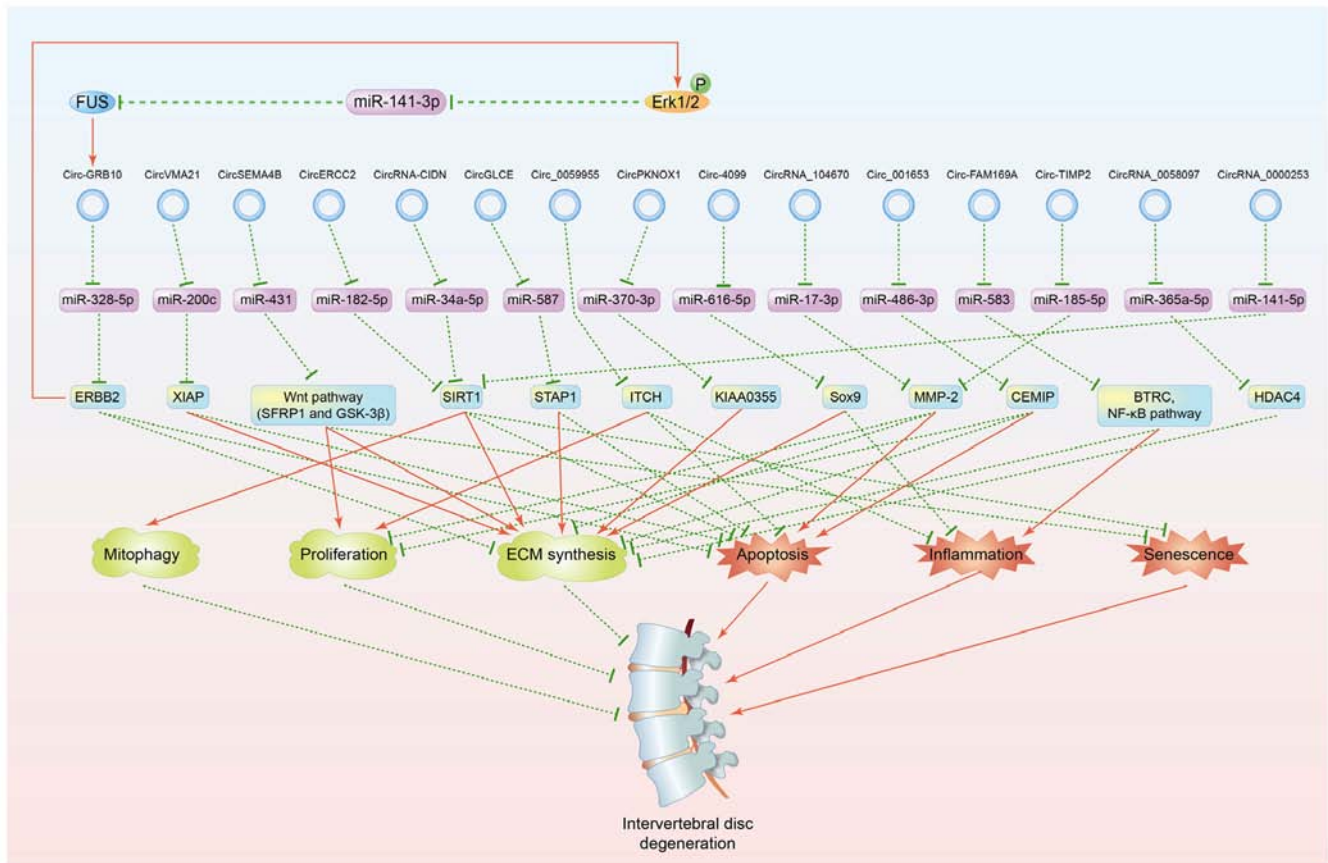
Figure 1. circRNAs involved in the regulation of IDD. Red solid lines indicate upregulation and green dashed lines represent downregulation. circRNA, circular RNA; miR, microRNA; P, phosphorylation; ECM, extracellular matrix; IDD, intervertebral disc degeneration; FUS, RNA-binding protein FUS; ERBB2, Erb-b2 receptor tyrosine kinase 2; XIAP, X linked inhibitor-of-apoptosis protein; SFRP1, secreted frizzled related protein 1; GSK-3β, glycogen synthase kinase-3β; SIRT1, silent mating type information regulation 2 homolog 1; STAP1, signal transducing adaptor family member 1; ITCH, itchy E3 ubiquitin protein ligase; Sox9, SRY-related high mobility group-box gene 9; MMP-2, matrix metalloproteinase 2; CEMIP, cell migration-inducing hyaluronan binding protein; BTRC, β-transducin repeat containing; NF-κB, nuclear factor-κB; HDAC4, histone deacetylase 4.

ECM metabolism by sponging miR-365a-5p and activating the expression of histone deacetylase 4 (HDAC4) in endplate chondrocytes induced by tension loading (Fig. 2).

We previously focused on the roles of exosome-transported circRNAs in IDD (37) and found that degenerative NPCs secreted more exosomes than the controls in NPC culture medium. Silencing circRNA_0000253, which was strongly upregulated in degenerative NPC exosomes, promoted ECM synthesis. Further investigations were performed both *in vitro* and *in vivo*, and indicated that exosome-transported circRNA_0000253 aggravates IDD via the miRNA-141-5p-SIRT1 axis (Fig. 3).

Collectively, circRNA-miRNA-mRNA axes play crucial roles in regulating ECM homeostasis.

3. Roles of circRNA in cell proliferation and apoptosis

The intervertebral disc, as the largest avascular tissue in the human body, is a cartilaginous structure with low cell density, despite the cells in the intervertebral disc being responsible for ECM synthesis and maintenance. IDD is characterized by a diminution in cell number and viability. Thus, cell proliferation and apoptosis are crucial to the pathogenesis of IDD (38,39). For example, it was found that circVMA21 overexpression attenuated cell apoptosis in NPCs after treatment with TNF-α and IL-1β by acting as an miR-200c sponge and upregulating

XIAP (23). Urban *et al* (40) clarified the strong association between nutrition and IDD. It has been demonstrated that loss of nutrient supply can lead to cell death and an increase in ECM degradation, and hence to IDD (41). Guo *et al* (26) reported that circ-GRB10 expression was significantly downregulated and miR-328-5p expression was significantly upregulated in IDD samples compared with normal controls. Upregulation of circ-GRB10 expression significantly suppressed cell apoptosis in NPCs after nutrient deprivation *in vitro*. Further experiments demonstrated that circ-GRB10 serves as a regulator of NPC survival by sequestering miR-328-5p activity and subsequently promoting the expression of ERBB2. Li *et al* (42) found that circ-FAM169A expression was strongly upregulated in IDD tissues. Dual luciferase reporter assays corroborated the direct binding among circ-FAM169A, miR-583 and Sox9. Although functional and rescue experiments were lacking, functional enrichment analyses suggested that the circ-FAM169A-miR-583 axis might be involved in ECM metabolism and NPC survival.

In our previous study, interfering with circRNA_104670 facilitated the proliferation and suppressed the apoptosis of NPCs, as evaluated by MTT assays and cell flow cytometry (31). Wnt/β-catenin signaling is involved in a multitude of biological processes, including cell proliferation, apoptosis and differentiation, embryonic development and tissue organization (43). It was reported that upregulation of circSEMA4B expression decreased
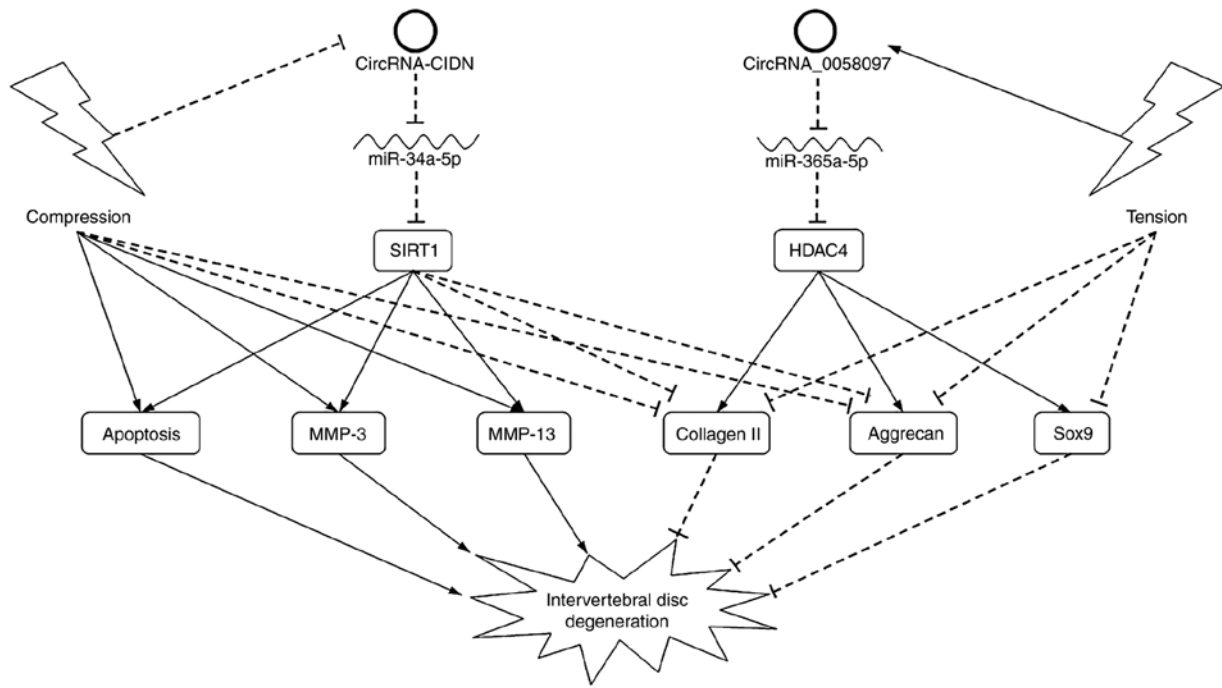
Figure 2. Mechanical loading induces intervertebral disc degeneration via circRNAs. circRNA, circular RNA; miR, microRNA; HDAC4, histone deacetylase 4; SIRT1, silent mating type information regulation 2 homolog 1; MMP, matrix metalloproteinase; collagen II, type II collagen; Sox9, SRY-related high mobility group-box gene 9.

proliferation and activated senescence through the miR-431-Wnt pathway in NPCs after treatment with IL-1β (24). circ_001653 inhibition promoted proliferation and suppressed apoptosis in NPCs via miR-486-3p-CEMIP (32). circRNA-CIDN overexpression inhibited apoptosis via the miR-34a-5p-SIRT1 axis in NPCs after compression loading (33). Our previous study showed that exosome-transported circRNA_0000253 could activate apoptosis while decreasing the proliferation of NPCs by competitively absorbing miRNA-141-5p (37). It was found that circGLCE inhibition increased the rate of apoptosis in NPCs after IL-1β treatment by acting as a sponge for miR-587 (28).

Notably, Kong et al (44) focused on the interaction between circRNA and target protein rather than the extensively studied classic ceRNA mechanisms. This study found that the expression level of hsa_circ_0059955 was significantly lower in IDD specimens than that in control specimens. Knockdown of hsa_circ_0059955 inhibited proliferation and induced apoptosis and G_0/G_1 phase arrest in NPCs. The expression of itchy E3 ubiquitin protein ligase (ITCH) was negatively correlated with hsa_circ_0059955. Forced expression of ITCH counteracted the suppression of NPC proliferation induced by hsa_circ_0059955 inhibition. These results suggested the existence of the hsa_circ_0059955-ITCH axis. However, further evidence verifying the direct binding between hsa_circ_0059955 and the ITCH protein is needed to make the hypothesis more convincing.

Thus, the aforementioned studies suggest that different circRNAs could exert opposing effects on NPC proliferation and apoptosis through ceRNA mechanisms.

4. Roles of circRNA in inflammation

Inflammatory mediators and signaling pathways are recognized as major contributors to the onset and development of IDD.

Elevated levels of inflammatory molecules have been detected in IDD tissues compared with those in healthy controls (45). Furthermore, ICs, particularly TNF-α and IL-1β, have been demonstrated to stimulate and deteriorate the progression of IDD (46). Thus, treatment of NPCs with ICs has been widely applied to construct in vitro IDD models (23,24,28,30,35). The nuclear factor-κB (NF-κB) signaling pathway is closely implicated in numerous complex biological processes, such as immunity and inflammatory responses. There are two distinct pathways leading to the activation of the NF-κB signaling cascades. The canonical pathway is mediated by nuclear translocation of RelA (p65), c-Rel and p50, while the non-canonical pathway is mediated by nuclear translocation of p52 and RelB (47). It has been identified that the NF-κB signaling pathway is a master regulator of inflammation and catabolism in IDD (48).

In the study by Wang et al (30), an IDD NPC model was generated by TNF-α treatment, and a higher expression level of circ-4099 was observed. When pathway inhibitors of the mitogen-activated protein kinase (MAPK) and NF-κB pathways were applied, the upregulation of circ-4099 expression induced by TNF-α was reversed, indicating that TNF-α enhanced circ-4099 expression via the MAPK and NF-κB pathways. Moreover, circ-4099 overexpression significantly decreased the expression level of IL-1β, TNF-α and prostaglandin E2 in NPCs, while miR-616-5p mimics could impair the anti-inflammatory effects of circ-4099.

As a component of the Skp-Cullin-F-box-containing E3 ubiquitin ligase complex, BTRC recognizes the NF-κB inhibitor IκBα and precursor p100 for proteasomal degradation and processing, respectively. Therefore, BTRC plays an important role in both the canonical and non-canonical NF-κB activation pathways (49). Guo et al (34) reported that circ-FAM169A was
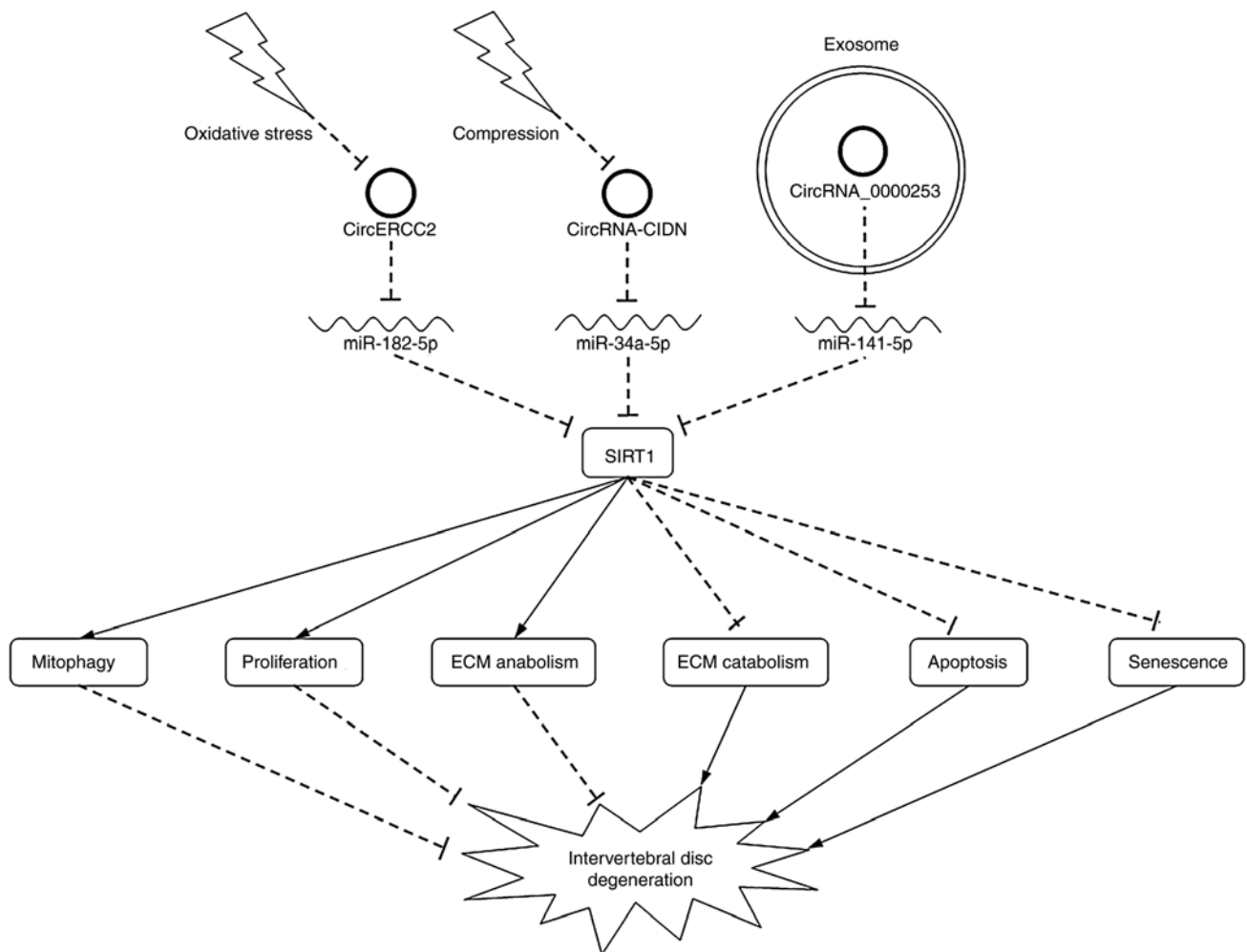
Figure 3. SIRT1 plays a protective role in intervertebral disc degeneration. circRNA, circular RNA; miR, microRNA; P, phosphorylation; ECM, extracellular matrix; SIRT1, silent mating type information regulation 2 homolog 1.

upregulated in IDD tissues. Knockdown of circ-FAM169A not only alleviated ECM degradation but also inhibited IL-1β, TNF-α and IKBα expression, while circ-FAM169A overexpression exhibited the opposite effects. Silencing BTRC could reverse the adverse effects of circ-FAM169A. In addition, luciferase reporter assays confirmed the direct binding among circ-FAM169A, miR-583 and BTRC.

5. Roles of circRNA in senescence

Cellular senescence is characterized by irreversible proliferative arrest in response to multiple stresses, such as telomere dysfunction, DNA damage, organelle stress and oncogene activation (50). A growing body of evidence has indicated that cellular senescence participates in the pathological process of a number of chronic age-associated diseases, including IDD (51-54). To date, the detection of senescent cells depends on a combination of various markers, including senescence-associated-β-galactosidase (SA-β-gal), aberrant cell morphology, the nucleoside analogs p21 and p16, nuclear senescence-associated heterochromatin foci and components of the senescence-associated secretory phenotype (55). Previous studies identified that circSEMA4B and circERCC2 both inhibited cellular senescence, as determined using SA-β-gal staining, in NPCs

stimulated by IL-1β and tert-butyl hydroperoxide (TBHP), respectively (24,25). Hence, circRNAs could affect NPC senescence through miRNA-mRNA pathways.

6. Roles of circRNA in mitophagy

Mitochondria are double membrane-enclosed organelles, playing pivotal roles in energy production, reactive oxygen species (ROS) generation and cell death (56). Mitochondrial dysfunction is associated with the occurrence and development of various diseases. Therefore, dynamic and relatively stable control of mitochondrial quantity and quality is crucial for preserving the physiological functions of cells (57). Mitophagy, a special form of autophagy, is a conserved and essential process to maintain mitochondrial homeostasis by selectively eliminating dysfunctional mitochondria (56).

The PTEN induced kinase 1 (PINK1)/Parkin-mediated mitophagy pathway is a classical and well-studied pathway in degenerative diseases (58). Wang et al (59) discovered that PINK1 expression was upregulated in IDD tissues. NPCs were treated with H_2O_2 to induce oxidative stress, which aggravated mitochondrial dysfunction and cellular senescence, and activated mitophagy in NPCs. Silencing PINK1 enhanced oxidative stress-induced NPC senescence by inhibiting

PINK1/Parkin-mediated mitophagy. Zhang *et al* (60) reported that Parkin expression was elevated both in IDD specimens and NPCs stimulated by TNF-α. Knockdown of Parkin increased mitochondrial damage, ROS production and apoptosis in NPCs, while upregulating Parkin expression alleviated the progression of IDD via Parkin-mediated mitophagy. Wang *et al* (61) revealed that SIRT1 attenuated ROS accumulation, mitochondrial injury and cellular senescence through PINK1-dependent mitophagy in NPCs treated with high-magnitude compression. Therefore, mitophagy appears to be closely correlated with IDD development.

Hirayama disease is a special neurological disorder, predominantly affecting male adolescents. Thus, non-degenerative NP tissues could be obtained from patients undergoing anterior cervical discectomy and fusion due to this disease (62). We previously showed that circERCC2 expression was significantly downregulated in IDD tissues compared with that in Hirayama disease samples (25). Forced expression of circERCC2 decreased the rate of apoptosis and activated mitophagy, which was evaluated by detecting the key markers for mitophagy (PINK1, Parkin, p62 and LC3II/I ratio), in NPCs induced by TBHP *in vitro*. The direct binding between circERCC2 and miR-1825p, and between miR182-5p and SIRT1 was confirmed by dual-luciferase assays. Further analysis was performed and determined that circERCC2 alleviates IDD by regulating NPC apoptosis and mitophagy through the miR-182-5p-SIRT1 axis.

7. Limitations and future directions

Over the past few years, a growing number of studies have shown that numerous circRNAs can contribute to the development and progression of IDD, particularly NPC phenotypic modulation (Fig. 1). circRNAs are abundant, highly conserved and stable, with tissue- and development-specific expression patterns. Therefore, the in-depth and reliable elucidation of the association between circRNAs and IDD will eventually facilitate the establishment of novel diagnostic and treatment methods.

However, there are still some limitations in the available studies. First, to the best of our knowledge, 17 studies have been published that investigated the potential roles of circRNAs in IDD. Of these, 15 mainly focused on ceRNA mechanisms, indicating that circRNAs exert biological functions by acting as miRNA sponges, and established circRNA-miRNA-mRNA axes. Notably, Kong *et al* (44) attempted to study the interaction between circRNA and target protein instead of classic ceRNA mechanisms. The results suggested that hsa_circ_0059955 inhibition negatively regulated NPC survival via the ITCH protein. However, this finding was not confirmed, as experiments verifying the direct binding between hsa_circ_0059955 and ITCH protein were not conducted. Notably, circRNAs not only sponge and sequester proteins, but also act as protein scaffolds to mediate the formation of circRNA-protein complexes and the cellular localization of specific proteins (9). Notably, although circRNAs are generally considered to be non-coding, as they lack 5' caps and polyadenylated tails that are essential for translation initiation, some circRNAs can function as templates for protein synthesis through cap-independent translation driven by internal ribosome entry sites or N6-methyladenosine (9).

Accordingly, future studies are required to discover other functional mechanisms of circRNAs in IDD in addition to their serving as miRNA sponges.

Second, most of the present studies concentrated on the downstream mechanisms of circRNAs, but the question of what causes the aberrantly altered expression of circRNAs in IDD has been less discussed. Notably, studies by Guo *et al* (26,27) established a complex signaling circuitry of circ-GBR10 rather than a linear pathway in IDD. It was found that an RNA-binding protein, FUS, was an upstream regulator of circ-GRB10. RNA immunoprecipitation further confirmed the direct interaction between circ-GRB10 and the FUS protein. Combining the results of two published studies by Guo *et al* (26,27), a circ-GBR10-miR-328-5p-ERBB2-Erk1/2 phosphorylation-miR-141-3p-FUS loop was constructed. In the future, it will be worthwhile to fully investigate circRNAs with their upstream regulators and downstream targets to establish circRNA networks. Further elucidation of the crosstalk among circRNAs can help us to recognize the key nodes to improve diagnosis and treatment.

Third, IDD is an extremely complex process. Studies tend to focus on NPCs, as they are the main type of cells in the NP and are responsible for ECM production. However, IDD also includes the deterioration of both AF and CEPs. Xiao *et al* (36) focused on endplate chondrocytes instead of NPCs and demonstrated that inhibition of circRNA_0058097 could exert positive effects on endplate chondrocyte degeneration via the miR-365a-5p-HDAC4 axis. More studies are needed to reveal how circRNAs affect the IDD process by regulating the functions of cells in AF and CEPs. Furthermore, numerous studies simulated IDD by culturing NPCs with ICs, such as IL-1β and TNF-α. In addition to ICs, oxidative stress, nutrient deprivation and mechanical loading are crucial factors inducing IDD (46,63-65). Xiang *et al* (33) indicated that circRNA-CIDN alleviated IDD induced by compression through the miR-34a-5p-SIRT1 axis. Thus, more research should be performed in different IDD models. In addition, proliferation, apoptosis, inflammation and ECM metabolism have been extensively studied. Only two studies described senescence (24,25), and one study discussed mitophagy in IDD (25). Therefore, the important phenotypes mediated by circRNAs, including cellular senescence, autophagy, mitophagy and ferroptosis, in IDD have not been sufficiently illuminated (50,57,66,67).

Recently, exosome-transported circRNAs have attracted increasing attention from scientists. Exosomes are small membrane vesicles that originate from multivesicular endosomes by inverse budding. Secreted by most eukaryotes, exosomes can carry various molecules, including lipids, proteins and nucleic acids, from one cell to another (68). It was previously noted that exosomal circRNA_0000253 can aggravate the progression of IDD via the miRNA-141-5p-SIRT1 axis (37), and the roles of exosome-transported circRNAs have become a hot topic in the research field of cancer (69). Therefore, it is essential to further determine whether these novel circRNAs are involved in the pathological process of IDD.

8. Conclusion

In conclusion, the latest evidence has confirmed that circRNAs play crucial roles in ECM synthesis and decomposition,

inflammation, cellular proliferation, apoptosis, senescence and mitophagy, mainly by functioning as sponges for miRNAs in the pathogenesis of IDD.

Availability of data and materials

Not applicable.

Authors' contributions

ZL, FZ and JJ were responsible for the conceptualization of the study. FZ, JJ and FL were responsible for the study design, and ZL, FL, XM and XX for the search strategy, article inclusion and interpretation. All authors were involved in original draft preparation. ZL, FZ and JJ were responsible for reviewing and editing the manuscript, and FZ and JJ supervised the project. All authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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