

Emerging role of circular RNA in intervertebral disc degeneration: Knowns and unknowns (Review)

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Abstract. Lower back pain (LBP) is one of the predominant factors contributing to dyskinesia and remains a serious social and economic burden worldwide. Intervertebral disc degeneration (IDD) is the leading cause of LBP; the existing IDD treatments cannot completely prevent IDD. Circular RNAs (circRNAs) are non-coding RNAs resulting from back-splicing with unique structural characteristics and functions. Accumulating evidence suggests that circRNAs are involved in the pathological process of IDD and modulate a range of IDD-related genes or proteins. However, the underlying circRNA-mediated regulatory mechanisms remain poorly understood. The aim of the present review is to describe the current understanding of circRNA characteristics, classification, biogenesis and function in relation to its specific roles in IDD. Additionally, the limitations on the current knowledge in the field and the future direction of IDD-related research are also discussed.

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1. Introduction

Lower back pain (LBP) is one of the predominant factors contributing to dyskinesia and the second most common cause of hospital visits, leading to a serious social and economic burden worldwide (1,2). Symptomatic intervertebral disc degeneration (IDD) is the most frequent cause of LBP (1,3). Although several factors contribute to IDD, genetic factors are the leading cause (4). The nucleus pulposus (NP) is located in the center of the intervertebral disc (ID) (5); it is the largest avascular tissue in the body and lacks blood oxygenation, which limits its self-repairing ability (6). Existing IDD treatments are not satisfactory and cannot fully recover ID function (7). Non-coding RNA (ncRNA) generated by gene back-splicing can regulate gene post-transcriptional modification to modulate disease development (8). Frapin *et al* (9) described the pathological process of IDD in detail and inferred that metabolic dysregulation of the extracellular matrix (ECM) in the ID microenvironment was predominantly involved in the pathogenesis of IDD. The various types of IDD-related genes or protein expression disorders can contribute to the synthesis and catabolic imbalance of ECM, giving rise to the alteration of ID morphology, physics and mechanics, leading to ID function loss, thereby triggering IDD (9-11). Gene therapy uses viruses and other vectors to carry ncRNAs formed by genes or genes to target ID, which can reverse or block the pathological process of IDD and recover ID function at the genetic level (10). In view of the aforementioned reasons, gene-based diagnostic and treatment strategies are critical measures for IDD management.

IDD-related genes or proteins can be divided into protective factors and catabolic factors. The former includes hypoxia-inducible factor-1 α (HIF-1 α), vascular endothelial growth factor (VEGF), collagen type II (COL2), aggrecan (ACAN), SRY-related high mobility group box 9 (SOX9) and a series of antiapoptotic proteins, whereas the latter includes

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matrix metalloproteinases (MMPs), disintegrin and ADAM metalloproteinases with thrombospondin type 1 motifs (ADAMTSs), interleukin (IL)-1 β , tumor necrosis factor- α (TNF- α) and a number of proapoptotic proteins (4,9-11).

Previous studies have suggested that ncRNAs, including microRNAs (miRNAs/miRs) and circular RNAs (circRNAs), serve a crucial role in the occurrence and progression of IDD (12-17). In particular, circRNAs mediate NP cell (NPC) apoptosis and regulate the expression of inflammatory cytokines, MMP, ADAMTS, various apoptosis-related proteins and key components of the ECM, such as COL2 and ACAN, which serve a role in the pathogenesis of IDD (12-17).

The present article provides an up-to-date review of circRNA characteristics, classification, biogenesis and function, with particular emphasis on the potential future directions of IDD-related research. Additionally, the limitations of current research are also discussed.

2. Characteristics

CircRNAs are a type of ncRNA with high thermal stability that were first discovered in plant-infecting virions in 1976 by Sanger *et al.* (18). Although circRNAs have been identified in different species (19-22), these molecules were not initially extensively studied. However, circRNAs were later found to exert a previously unrecognized role in a wide spectrum of human diseases, owing to the rapid development of next-generation sequencing technology (23,24). Compared with linear RNAs, covalently closed circRNAs have unique characteristics and biological functions without 5' to 3' polarity or a polyadenylated tail (25-27). They are predominantly located in the cytoplasm, abundantly expressed, conserved, highly stable and exonuclease-resistant (28-31). In addition, circRNAs are expressed in a tissue- and time-specific manner (31,32). They can also be carried in exosomes and have potential applications as markers for disease diagnosis (33,34). Direct back-splicing and exon skipping are the main pathways of circRNA synthesis (23,27).

3. Classifications

Currently, seven types of circRNAs have been identified according to the type and quantity of the parental gene the circRNAs originate from. These include exonic circRNA (ecircRNA), intronic circRNA (ciRNA), tRNA intronic circRNA (tricRNA), exon-intron circRNA (eiciRNA), read-through circRNA (rt-circRNA), fusion circRNA (f-circRNA) and mitochondria-encoded circRNA (meccirRNA). The circularization of at least one intron or one exon from a single gene gives rise to ciRNAs and ecircRNAs, respectively, whereas the formation of eiciRNA is based on the cyclization of at least an exon and an intron (24,26) (Fig. 1). TricRNA is a special type of circRNA synthesized through a pre-tRNA intron splicing mechanism (35). Rt-circRNA is the result of circularization of two exons from two different genes (30). F-circRNA is synthesized from the transcribed exons of several nuclear genes as a result of chromosomal translocation (36). Lastly, meccirRNA is produced from mitochondrial genes (37). EcircRNAs are the most common type of circRNA.

4. Biogenesis and functions

The biological functions of circRNAs depend on their type and cellular location. In the nucleus, synthetic circRNAs can modulate gene transcription (30,38) and alternative splicing (39). For example, eiciRNAs circ-EIF3J and circ-PAIP2 are largely localized in the nucleus and can interact with U1 small nuclear ribonucleoprotein and RNA polymerase (Pol) II to promote the transcription of their host genes (38). The ciRNA ci-ankrd52 positively regulates the function of RNA Pol II to modulate the transcription of its parental gene (ANKRD52), predominantly converging to the cell nucleus (39). Increased synthesis of circ-Mbl may repress the transcription of its parental gene (40).

After synthesis, ecircRNAs are transferred from the nucleus to the cytoplasm. As shown in Fig. 1, the synthesis of mature miRNAs involves a range of processing steps. First, miRNA genes are transcribed into pri-miRNAs, and then processed into pre-miRNAs in the nucleus, pre-miRNAs are then transported into the cytoplasm via the nuclear export protein Exportin5 to produce mature miRNAs (41). Subsequently, mature miRNAs directly interact with the 3'UTRs of target mRNAs, thereby inhibiting mRNA translation or degrading mRNA (42). EcircRNAs that are primarily located in the cytoplasm can modulate the expression of their parental genes (43,44), sponge miRNAs by acting as competitive endogenous RNAs (ceRNAs) (25,31,43-46), attach miRNAs (47,48), interact with or sponge RNA-binding proteins (RBPs) (25,31,49-52), encode proteins (25,31,43,53-55) and modulate protein translation (56-58) (Fig. 1).

The crosstalk between circRNAs and miRNAs is complex. CircRNAs commonly inhibit miRNA expression through a 'sponging' mechanism (25,31,41-44). Additionally, circ-CSNK1G3 and ci-RS-7 positively regulate the levels of miR-181b/d and miR-7, respectively (45,46). Conversely, miR-200b negatively modulates the expression and function of circRNA-000839 (59). Whether miRNA, in turn, can regulate circRNA expression remains unknown. Moreover, the possibility that circRNAs also interact with each other has yet to be demonstrated. Furthermore, cytoplasmic circRNAs also modulate parental gene expression. For example, F-box and WD repeat domain-containing 7 (FBXW7), a well-known tumor suppressor gene, encodes the FBXW7 protein and circ-FBXW7. Circ-FBXW7 regulates the mRNA and protein levels of FBXW7 to repress the expression of c-Myc in a miRNA-dependent manner (41). Another study suggests that circ-filamin-binding LIM protein 1 (FBLIM1) positively regulates the expression of the FBLIM1 gene by sponging miR-346 (42).

CircRNAs regulate the activity and function of proteins in several ways, including via sponging, as a protein scaffold, by encoding proteins and modulating protein translation (Fig. 1). Circ-forkhead box O3 (Foxo3) can bind to both P21 and cyclin-dependent kinase 2 (CDK2) to generate ternary complexes that enhance the inhibitory effect of P21 on CDK2 (47). Circ-Foxo3 also functions as a protein scaffold, stabilizing Foxo3 protein expression by interacting with mouse double minute 2 (Mdm2) and P53 and inhibiting Mdm2-induced Foxo3 ubiquitination (50). Additionally, circ-Foxo3 inhibits the nuclear translocation of transcription factors, including HIF-1 α , thereby inhibiting their

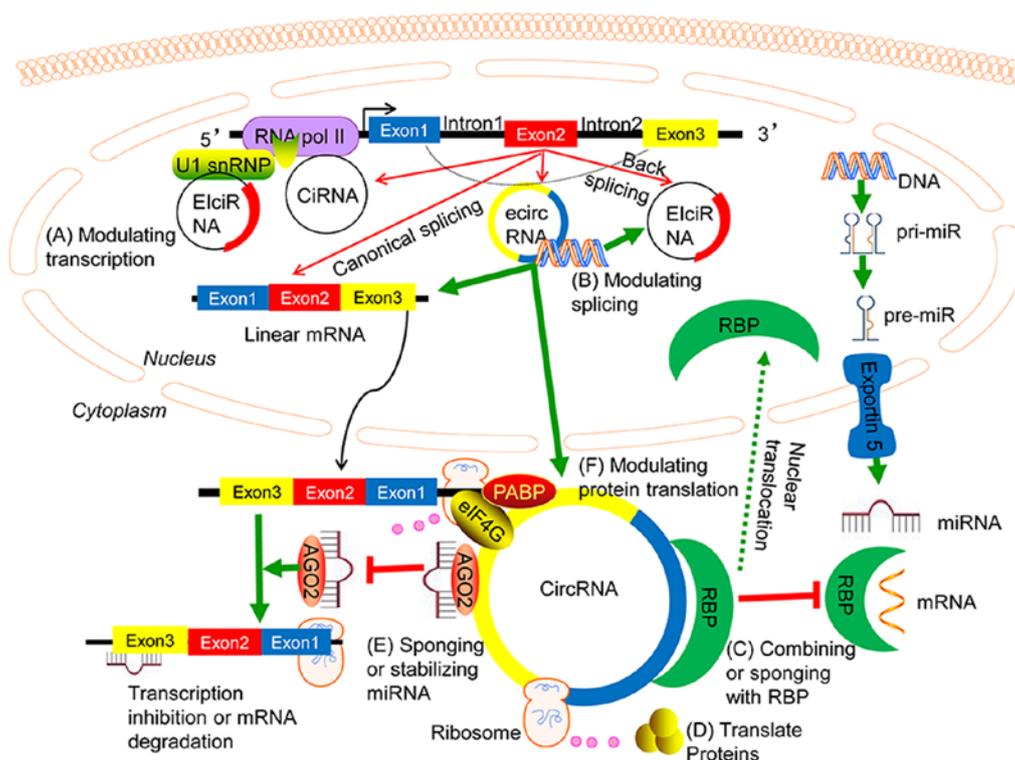


Figure 1. Biogenesis and function of circRNAs. In the nucleus, the circularization of at least one intron or one exon from a single gene gives rise to ciRNAs and ecircRNAs, respectively, whereas the formation of eiciRNA is based on the cyclization of at least one exon and one intron. CiRNAs or eiciRNA, mostly enriched in the nucleus, can modulate gene transcription and alternative splicing. EcircRNAs, which are primarily located in the cytoplasm, can modulate the expression of their parental genes, sponge miRNAs, interact with or sponge RBP, translate proteins and modulate protein translation. EcircRNAs positively regulate the expression of their target genes via sponging miRNAs to relieve the inhibitory effect of miRNAs on target genes. CircRNA, circular RNA; RBP, RNA-binding protein; ciRNA, intronic circRNA; ecircRNA, exonic circRNA; eiciRNA, exon-intron circRNA; miRNA, microRNA.

functions (49). Circ-ZKSCAN1 sponges fragile X mental retardation protein, preventing it from binding to its downstream target mRNA, cell cycle and apoptosis regulator 1, thereby attenuating the malignant biological behavior of hepatocellular carcinoma (HCC) through the Wnt signaling pathway (48). RBPs also regulate the formation of circRNAs. For example, RNA-binding motif protein 3 (RBM3) increases stearyl-CoA desaturase-circ-RNA 2 synthesis to promote HCC cell proliferation (60), and Quaking 5 may promote the synthesis of circ-ZKSCAN1 (48). In addition, some circRNAs function through the proteins they encode. Circ- β -catenin encodes a 370-amino-acid β -catenin, which inhibits glycogen synthase kinase 3 β (GSK3 β)-mediated β -catenin degradation and activates the Wnt/ β -catenin pathway in HCC (51). Circ-protein phosphatase 1 regulatory subunit 12A (PPP1R12A)-73aa (a protein encoded by circ-PPP1R12A), but not circPPP1R12A itself, accelerates colon cancer growth and metastasis (52). Moreover, circ-FBXW7 encodes the FBXW7 185-amino-acid protein (FBXW7-185aa), and the synergistic action of FBXW7 and FBXW7-185aa stabilizes c-Myc and promotes oncogenesis and tumor progression (42). Lastly, partial circRNAs modulate protein translation in a protein-dependent manner. CircRHOT1 regulates the translation of nuclear receptor subfamily 2 group F member 6 (NR2F6) by activating Tat-interacting protein of 60 kDa to the *Nr2f6* gene promoter (54). Circ-YY1-associated protein 1 (YAP) regulates the initiation efficiency of YAP protein translation by interacting with *Yap* mRNA and the proteins eIF4G and PABP (55). More recently, Sun *et al* (58)

reported that circMYBL2 could facilitate fms-related receptor tyrosine kinase 3 (FLT3) protein translation efficiency by recruiting polypyrimidine tract-binding protein 1 to bind to *Flt3* mRNA.

Recently, Chen *et al* (47) suggested that ~90% of circRNAs have an independent regulatory role in cell proliferation, compared with their linear counterparts. Nevertheless, certain circRNAs have functions that are similar to linear RNAs (61,62). Peroxisome proliferator-activated receptors and their associated circRNA, circ-5379-6, both suppress tumor progression (61). Yao *et al* (62) also reported that circ-ZKSCAN1 and its parental gene both inhibited cell growth through distinct signaling pathways.

5. Specific roles of circRNA in IDD

Biofunctions of circRNA in IDD. A growing body of evidence suggests that circRNAs are extensively involved in a multitude of chronic diseases, including osteoarthritis (63) and cancer (64), as well as cardiovascular (65), neurodegenerative (66,67) and immunological (68,69) diseases. However, the role of circRNA in IDD remains unclear. To the best of our knowledge, only nine upregulated and nine downregulated circRNAs have been identified in degenerative NP samples compared with normal NP samples, and the functions of 12 of these dysregulated circRNAs remain fairly poorly understood (70,71). Using microarray data from the Gene Expression Omnibus (GEO) database, Zhu *et al* (72)

predicted three circRNA-mediated regulatory pathways in IDD, namely, circRNA-102348/miR-185-5p/TGFB1/FOS, circRNA-102399/miR-302a-3p/HIF1A and circRNA-100086/miR-509-3p/MAPK1. However, further investigation is needed to elucidate their potential role in IDD (72). Moreover, the detailed biofunctions of circRNAs that have been identified are listed in Table I and Fig. 2. The upregulated circRNAs except circ-0004099 contribute to the occurrence and progression of IDD, whereas circ-0004099 and all downregulated circRNAs function as IDD repressors. CircRNAs act as IDD repressors or enhancers through the regulation of several pathological processes, including NPC apoptosis, proliferation, mitophagy and senescence, as well as the dysregulation of MMP, ADAMTS, inflammatory cytokines and ECM expression.

The five described IDD repressors have diverse functions: i) Circ-semaphorin 4B (SEMA4B) enhances NPC proliferation; ii) circ-SEMA4B and circ-excision repair cross-complementation group 2 (ERCC2) suppress NPC senescence; iii) circ-growth factor receptor bound protein 10 (GRB10), circ-vacuolar ATPase assembly factor VMA21 (VMA21) and circ-ERCC2 suppress NPC apoptosis; iv) circ-SEMA4B, circ-0004099, circ-VMA21 and circ-ERCC2 promote ECM synthesis; v) circ-VMA21 and circ-ERCC2 suppress MMP or ADAMTS expression; vi) circ-ERCC2 facilitates mitophagy; and vii) circ-0004099 inhibits IC secretion (12-17). The only known IDD enhancer, circRNA-104670, not only represses NPC proliferation and the synthesis of ECM components, but also promotes NPC apoptosis and MMP2 expression (15). These abnormally expressed circRNAs mediate pathological processes through several signaling pathways, including apoptosis-related pathways and ECM-related pathways.

Circ-VMA21-mediated IDD repression. Circ-VMA21 was the first identified IDD-related circRNA (12), providing invaluable insight into the modulation of IDD pathogenesis by circRNAs. Circ-VMA21 is downregulated in degenerative NP samples from patients with IDD compared with NP samples from controls (12). Similarly, the expression of circ-VMA21 is reduced in NPCs treated with both TNF- α and IL-1 β (12). The progression of IDD is associated with the aberrant expression of circ-VMA21 in degenerative and normal NP samples. More specifically, circ-VMA21 blocked the progression of IDD, and its downregulation limited its protective effect. Circ-VMA21 serves a protective role in human NPCs and rat NP tissues predominantly through apoptosis-related pathways and the ECM components metabolism-related pathways.

Circ-VMA21 positively modulates X linked inhibitor-of-apoptosis protein (XIAP) and represses the expression of caspase (CASP) family members (CASP-3, CASP-7 and CASP-9), as well as a number of degrading metabolic enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5). Circ-VMA21 also promotes the expression of ECM components, including COL2 and ACAN, by sponging miR-200c (12,73). Luciferase reporter and RNA pull-down assays confirmed that circ-VMA21 has five effective binding sites in miR-200c (12,73).

Pfirrmann classification is the most common method used for the evaluation of IDD severity, according to magnetic resonance imaging grade (74). Circ-VMA21 markedly

decreased the Pfirrmann grade of IDD following injection into rat IDs. Altogether, these studies suggested that the circ-VMA21/miR-200c/XIAP axis may be involved in the regulation of IDD pathogenesis, providing novel therapeutic targets for IDD.

IDD repressor circ-GRB10. Lan *et al* (75) analyzed the microarray data of human lumbar IDD and uploaded it into the GEO database. Our previous study identified three abnormally expressed circRNAs by analyzing circRNA microarray data from the GEO database, of which two were upregulated (circ-FAM169A and circ-SETD2) and one was downregulated (circ-GRB10) (13). The expression of circ-GRB10 was downregulated in 20 degenerative NP samples from patients with IDD undergoing discectomy compared with 20 nondegenerative NP samples from patients with fresh traumatic lumbar fracture (13). Mechanistically, the circ-GRB10-mediated pathological process of IDD is miR-328-5p-dependent. Functionally, circ-GRB10 acts as an IDD repressor of NPC apoptosis under nutrient deprivation conditions by sequestering miR-328-5p and promoting Erb-B2 receptor tyrosine kinase 2 (ERBB2) expression in NPCs. Thus, circ-GRB10 downregulation could decrease NPC survival, leading to IDD onset and progression.

IDD repressor circ-0004099. Wang *et al* (14) categorized patients with IDD according to the Pfirrmann classification criteria (74). Patients with Pfirrmann grade I/II were assigned to a nondegenerative group, whereas those with Pfirrmann grade IV/V constituted the degeneration group. The authors collected six degenerative NP samples from the patients of the degenerative group who were undergoing spinal surgery and six nondegenerative NP samples from the patients of the nondegenerative group with vertebral fracture or scoliosis (13,14). Using circRNA microarray, circRNA expression profiles in the twelve samples were examined (14). Circ-0004099 was the most frequently upregulated circRNA in the degenerative samples (14). Moreover, circ-0004099 expression was increased in NPCs in a dose and time-dependent manner following treatment with TNF- α . It was also demonstrated that this effect was mediated by MAPK and the NF- κ B signaling pathways (14). Notably, circ-0004099 overexpression enhanced (rather than repressed) the expression of Sox9 and ECM proteins, such as ACAN and COL2. Circ-004099 also repressed (rather than enhanced) proinflammatory cytokine secretion (TNF- α , IL-1 β and prostaglandin E2), and these changes were reversed by miR-616-5p mimic (14). Sox9 is a chondrocyte-specific transcription factor that promotes COL2 and ACAN synthesis (76,77). Wang *et al* (14) also confirmed that Sox9 was the direct target of miR-616-5p. In addition, luciferase reporter, RNA immunoprecipitation and RNA pull-down assays indicated that circ-0004099 could bind to miR-616-5p (14). Collectively, these research results reveal that the circ-0004099/miR-616-5p/Sox9 axis might play a protective role in IDD.

IDD repressor circ-SEMA4B. Consistent with Guo's method (13), Wang *et al* (16) also analyzed the same circRNA microarray data that was downloaded from the GEO database. Circ-SEMA4B expression was the most significantly downregulated circRNA in 45 IDD specimens and had

Table I. List of circRNAs involved in IDD.

Author, year	circRNA	Condition	Expression	Treatment	Spinal type	Pathway	Investigated process	ceRNA mechanism	(Refs.)
Guo <i>et al.</i> , 2018	circ-GRB10	Fracture vs. IDD disease	Downregulated in IDD disease	N/A	Lumbar	circ-GRB10/miR-328-5p/ERBB2	Inhibition of NPC apoptosis	IDD repressor	(13)
Cheng <i>et al.</i> , 2018	circ-VMA21	Fracture or scoliosis vs. degenerative spinal disease	Downregulated in IDD disease	TNF- α (5 ng/ml) + IL-1 β (5 ng/ml)	Lumbar	circ-VMA21/miR-200c/XIAP/CASP9/ECM	NPC apoptosis inhibition; ECM degradation; MMP3/13; and ADAMTS4/5 expression	IDD repressor	(12)
Wang <i>et al.</i> , 2018a	circ_0004099	Fracture or scoliosis vs. degenerative spinal disease	Upregulated in IDD disease	TNF- α (50 ng/ml)	Lumbar	TNF- α /MAPK/NF- κ B/circ_0004099/miR-616-5p/Sox9/ECM/TNF- α and IL-1 β	Sox9 activation; ECM synthesis; and TNF- α /IL-1 β secretion	IDD repressor	(14)
Wang <i>et al.</i> , 2018b	circ-SEMA4B	Mild vs. severe IDD disease	Downregulated in IDD disease	IL-1 β (10 ng/ml)	Lumbar	IL-1 β /circ-SEMA4B/miR-431/GSK-3 β /SFRP1/Wnt pathway	ECM generation; NPC proliferation; and suppression of NPC senescence	IDD repressor	(16)
Xie <i>et al.</i> , 2019	circ-ERCC2	Cervical spondylotic myelopathy vs. Hirayama disease	Downregulated in IDD disease	TBHP (100 μ M)	Cervical	circ-ERCC2/miR-182-5p/SIRT1	Inhibition of NPC apoptosis; senescence; mitophagy; COL2; degradation	IDD repressor	(17)
Song <i>et al.</i> , 2018	circRNA_104670	Cervical spondylotic myelopathy vs. Hirayama disease	Upregulated in IDD disease		Cervical	circRNA_104670/miR-17-3p/MMP2	and MMP13 expression Enhancement of NPCs apoptosis and MMP2 expression; suppression of NPC proliferation and COL2 synthesis	IDD enhancer	(15)

CircRNA, circular RNA; IDD, intervertebral disc degeneration; miR, microRNA; circ-VMA21, circ-vacuolar ATPase assembly factor 21; circ-SEMA4B, circ-semaphorin 4B; circ-ERCC2, circ-excision repair cross-complementation group 2; circ-GRB10, circ-growth factor receptor bound protein 10; MMP, matrix metalloproteinase; NPC, nucleus pulposus cell; ceRNA, competing endogenous RNA; ERBB2, ErbB2 receptor tyrosine kinase 2; XIAP, X linked inhibitor of apoptosis protein; CASPs, caspases; GSK-3 β , glycogen synthase kinase-3 β ; SFRP1, secreted frizzled-related protein 1; SIRT1, silent information regulator 1; COL2, collagen type II; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; TBHP, tert-butyl hydroperoxide.

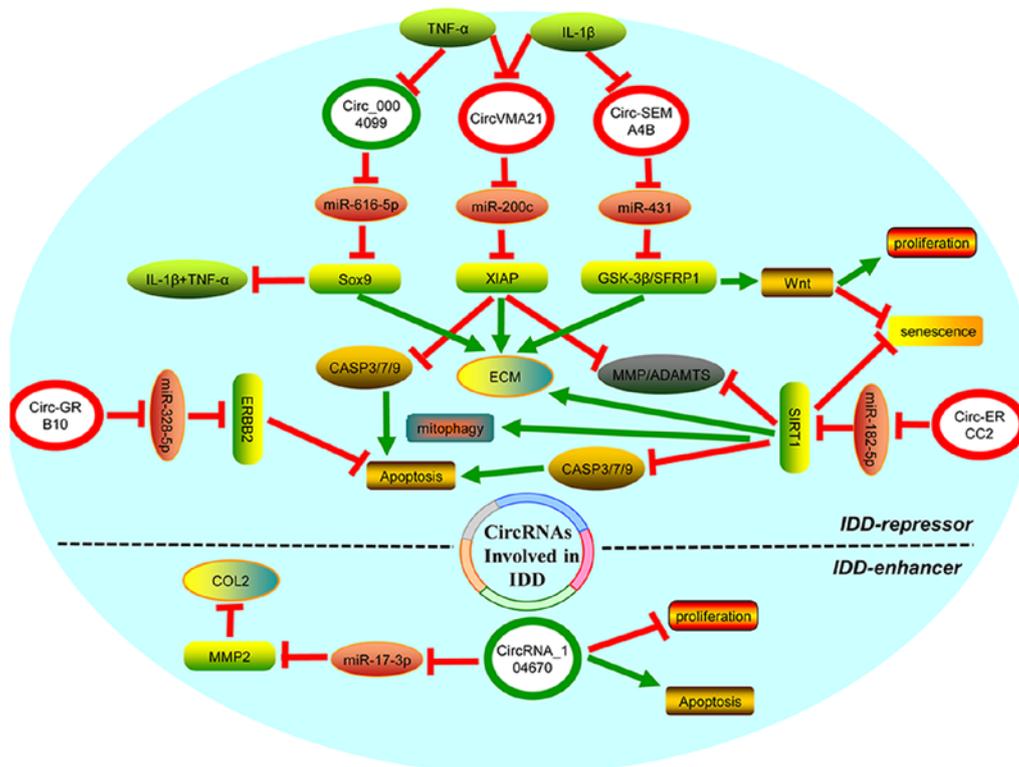


Figure 2. CircRNAs are involved in the regulation of IDD. In the upper half of the ellipse, circ-SEMA4B, circ-GRB10, circ-VMA21 and circ-ERCC2 were downregulated, whereas circ-0004099 was upregulated in IDD (green circle). All of them function as an IDD-repressor by regulating different miRNA-mRNA pathways, of which circ-SEMA4B, circ-0004099, circ-VMA21 and circ-ERCC2 promote the synthesis of key components of the ECM; circ-GRB10, circ-VMA21 and circ-ERCC2 suppress NPC apoptosis; circ-VMA21 and circ-ERCC2 suppress NPC senescence; circ-SEMA4B enhances NPC proliferation; circ-VMA21 and circ-ERCC2 suppress MMP or ADAMTS expression; circ-ERCC2 facilitates mitophagy; and circ-0004099 inhibits TNF- α and IL-1 β secretion. Additionally, TNF- α can inhibit circ-0004099 expression, IL-1 β can inhibit circ-SEMA4B expression, and both of them elevate the expression of circ-VMA21. In the lower half of the ellipse, circRNA-104670 was upregulated in IDD (green circle), which not only represses NPC proliferation and synthesis of ECM components, but also promotes NPC apoptosis and MMP2 expression via sponging miR-17-3p, thereby its acts as an IDD enhancer. CircRNA, circular RNA; IDD, intervertebral disc degeneration; circ-VMA21, circ-vacuolar ATPase assembly factor 21; circ-SEMA4B, circ-semaphorin 4B; circ-ERCC2, circ-excision repair cross-complementation group 2; circ-GRB10, circ-growth factor receptor bound protein 10; miR, microRNA; ECM, extracellular matrix; MMP, matrix metalloproteinase; NPC, nucleus pulposus cell; ADAMTS, ADAM metalloproteinases with thrombospondin type 1 motifs; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin 1 β .

a negative association with IDD severity, as based on the Pfirrmann grade (16). Notably, circ-SEMA4B promoted the synthesis of ECM components and NPC proliferation, while inhibiting NPC senescence under IL-1 β stimulation. It was also demonstrated that IL-1 β exerted these effects by inhibiting circ-SEMA4B expression (16).

Previous studies have demonstrated that the Wnt signaling pathway serves an important role in the regulation of NPC proliferation and senescence (78,79). For instance, circ-SEMA4B regulates the activation of the Wnt signaling pathway by sponging miR-431, the upstream regulator of two well-known Wnt signaling pathway inhibitors, GSK-3 β and secreted frizzled-related protein 1 (SFRP1) (16,80,81). Collectively, results from these studies suggest that circ-SEMA4B may be associated with the prognosis of patients with IDD and inhibits IDD development by regulating the miR-431/GSK-3 β /SFRP1 axis.

IDD repressor circ-ERCC2. Xie *et al* (17) analyzed Song's (15) microarray analysis of circRNAs and Lan's (75) microarray dataset (GSE67566) and demonstrated that circ-ERCC2 was the most frequently downregulated circRNA in degenerative NP tissue (15,74). Functional analyses also suggested that circ-ERCC2 modulated tert-butyl hydroperoxide-induced

NPC apoptosis (through CASP-3, CASP-7 and CASP-9), mitophagy (through PTEN-induced kinase 1, parkin, p62, and LC3II/I) and ECM structure (MMP13 and COL2) *in vitro* and *in vivo* (17). Fluorescence *in situ* hybridization and dual-luciferase assays demonstrated that circ-ERCC2 could bind to miR-182-5p (17).

Previous studies have indicated that silent mating type information regulator 2 homolog 1 (SIRT1) plays a significant role in mitophagy and apoptosis (82-84). SIRT1-small interfering (si)RNA inhibits NPC apoptosis and senescence. Moreover, this effect is suppressed by circ-ERCC2 and miR-182-5p inhibitor, suggesting that circ-ERCC2 exerts a protective effect on NPCs that is dependent on miR-182-5p (17).

IDD enhancer circRNA_104670. CircRNA-104670 is related to cervical IDD and is upregulated ~4.5-fold in degenerative tissues from patients with cervical spondylotic myelopathy compared with normal tissues from patients with Hirayama disease (15). Furthermore, circRNA-104670 may represent a diagnostic marker for IDD. In a previous study, receiver operating characteristic curve analysis indicated that the area under the curve value of circRNA_104670 was 0.96 and the expression of circRNA_104670 was positively associated with

Pfirrmann grade (15). Functionally, circRNA-104670 increased NPC apoptosis and suppressed NPC proliferation. Moreover, this circRNA also promoted MMP2 and repressed COL2 expression by sequestering miR-17-3p (15). Mice injected with circRNA_104670 siRNA presented lower Pfirrmann grades (15). Thus, circRNA-104670 may act as an IDD enhancer that regulates miR-17-3p and MMP2, leading to IDD progression.

6. Current limitations and future directions

All of the research on IDD-related circRNAs has shortcomings. Although a universal approach to NPC culture with inflammatory cytokine treatment and ID microenvironment stimulation has been developed, it does not account for the fact that different circRNAs have diverse affinities for various inflammatory cytokines. However, as highlighted in Table I, none of the investigations explain why they used IL-1 β or TNF- α or both. Recently, Shen *et al* (63) approached this problem by detecting the expression of circRNAs in the cells stimulated with IL-1 β , TNF- α or both.

As the NP is a hypoxic environment, the potential role of HIF-1 α in IDD has been reviewed previously. In the development of IDD, HIF-1 α activation is involved in the regulation of IDD-related gene or protein expression (9). Thus, HIF-1 α is a crucial transcriptional regulator of IDD. Increasing evidence suggests that HIF-1 α is a target of ncRNAs in several diseases (26,85,86). Nevertheless, hypoxia-related circRNA pathways in IDD are still poorly characterized.

Exosomes serve an important role in numerous physiological and pathological processes in various diseases (33,87,88). Various factors, including HIF-1 α , ncRNAs and proteins, among others, are present in exosomes (89). Thus, circRNAs contained within exosomes could serve as markers for diseases (33,87,88). Importantly, whether HIF-1 α affects MMP and the ECM remains unclear (9). Nevertheless, we cannot rule out the possibility of the existence of the circRNA/HIF-1 α /MMP/ECM axis in the exosomes of IDD. Further research is needed to elucidate this.

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) axis regulates numerous biological events, including cell proliferation, apoptosis, metastasis and metabolism (90). Growing evidence also indicates that circRNA-mediated regulation of the PI3K/AKT/mTOR axis serves an essential role in the pathogenesis of several diseases, such as hepatocellular carcinoma and kidney cancer (85,91,92). However, whether this holds true in IDD remains unclear. Recently, bioinformatics analysis predicted that circRNAs could regulate autophagy signaling pathways in IDD via sponging miRNAs (93). So, the role of circRNA-mediated autophagy in IDD cannot be ruled out and is a worthwhile research direction.

7. Conclusions

In conclusion, circRNAs function as ceRNAs to regulate the pathological process of IDD in a miRNA-dependent manner. However, circRNAs also have a number of other functions aside from their role as a ceRNA that have not been reported on in IDD. Whether circRNAs can sponge or interact with RBP, encode proteins, modulate protein translation and gene

expression in the context of IDD should also be addressed. Thus, understanding the biological role of circRNAs and their underlying molecular mechanism in the context of IDD would provide further insight into disease prevention strategies and contribute to the development of therapeutic targets for IDD.

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Availability of data and materials

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Authors' contributions

YL, SZ and PP contributed to the concept and the design of the review. YL wrote the manuscript. XW helped draft the manuscript and drew the figures. LD provided significant suggestions for the study. ZH searched the literature and collated important reference information. BX critically reviewed the manuscript. All authors read and approved the final manuscript.

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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