# Role of microRNAs in intervertebral disc degeneration (Review)

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**Abstract.** The incidence of lower back pain caused by intervertebral disc degeneration (IDD) is gradually increasing. IDD not only affects the quality of life of the patients, but also poses a major socioeconomic burden. There is currently no optimal method for delaying or reversing IDD, mainly due to its unknown pathogenesis. MicroRNAs (miRNAs/miRs) participate in the development of a number of diseases, including IDD. Abnormal expression of miRNAs in the intervertebral disc is implicated in various pathological processes underlying the development of IDD, including nucleus pulposus (NP) cell (NPC) proliferation, NPC apoptosis, extracellular matrix remodeling, inflammation and cartilaginous endplate changes, among others. The focus of the present review was the advances in research on the involvement of miRNAs in the mechanism underlying IDD. Further research is expected to identify markers for early diagnosis of IDD and new targets for delaying or reversing IDD.

**Contents**



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## 1. Introduction

Intervertebral disc (IVD) degeneration (IDD) is one of the main causes of low back pain (1-3). Low back pain is common, with ~80% of the population experiencing low back pain at different time points during their lifetime (4,5), and ~40% of all cases are caused by IDD (6). Low back pain caused by IDD costs ~70 billion euros annually worldwide (7). IDD not only affects the quality of life of the patients, but also poses a major socioeconomic burden. However, there is currently no effective and reliable treatment for IDD, mainly due to its unknown pathogenesis. Reversing or delaying the progression of IDD is particularly important for restoring the original physiological structure and function of the spine.

IDD has been widely considered as the result of 'wear and tear' due to aging and mechanical strain (8), but these factors have limited impact on the IVDs (9), and several studies have found that genetic factors account for 74% of all cases of IDD (10). MicroRNAs (miRNAs/miRs) are small endogenous non-coding RNAs that regulate gene expression at the post-transcriptional level (11), participating in a number of processes, including cell proliferation, differentiation and apoptosis, among others (12,13). miRNAs have also been closely linked to the process of disc degeneration, with several advances in related research (14-17). The aim of the present review was to focus on the progress of the research on the role of miRNAs in nucleus pulposus (NP) cell (NPC) proliferation and apoptosis, inflammation, extracellular matrix (ECM) remodeling and cartilaginous endplate (CEP) changes, and to discuss the pathogenic mechanisms and potential therapeutic prospects of miRNAs in the treatment of IDD.

## 2. miRNAs and IDD

The molecular pathological mechanism of IDD remains largely unclear. However, previous studies have shown that IDD is closely associated with apoptosis, cell proliferation, ECM degradation and inflammation (18,19). There is increasing evidence that miRNAs are involved in several aspects of cellular function, such as proliferation, apoptosis and inflammation, thereby regulating a series of pathophysiological changes that affect a number of processes. Several studies have reported significant changes in miRNA expression in degenerated IVD tissue (20-28), a number of which may be involved in the pathological process of IDD.

## 3. miRNAs and NPC proliferation

Young and healthy human IVDs contain two types of cells: Notochordal cells, which are vacuolated cells originating from the embryonic notochord; and NPCs. NPCs are less dense in healthy human IVDs and have specific distribution areas (29,30). One characteristic of IVD degeneration is the appearance of clusters of cells, particularly in damaged areas (31). The appearance of cell clusters is considered to be the result of abnormal proliferation of NPCs, which is closely associated with IVD degeneration (32). Proliferation is the basic life activity of cells and is affected by a number of factors. miRNAs regulate a variety of physiological activities and pathological processes, including cell proliferation, at the post-transcriptional level (12,33).

miR-21 is one of the most extensively investigated miRNAs. It is expressed in a variety of tissue types (34-36) and is involved in the regulation of cell proliferation (37). The expression of miR-21 in degenerated NP tissue is significantly higher compared with that in healthy NP tissue and is closely associated with the degree of IVD degeneration (14). Moreover, bioinformatics target prediction has indicated that PTEN may be the target of miR-21, that miR-21 inhibits PTEN expression by directly targeting its 3'-untranslated region, and this inhibition is eliminated by miR-21 binding site mutation (14). In addition, miR-21 overexpression-mediated cell proliferation and increased cyclin D1 expression were almost completely blocked by the Akt inhibitor, Ly294002 (14). In conclusion, the abnormal upregulation of miR-21 in IVDs may target PTEN, which is involved in the abnormal proliferation of NPCs through derepressing the Akt pathway (14). This suggests that the miR-21 and PTEN/Akt pathways may be potential targets for inhibiting the abnormal proliferation of NPCs. In addition, miR-21 inhibitors can inhibit the expression of hypoxia-inducible factor-1α and VEGF in the annulus fibrosus (AF) and NP, and inhibit NPC apoptosis (15). Furthermore, miR-21 may promote the proliferation of NPCs via targeting programmed cell death 4 (16). Upregulation of miR-21 also increases the expression of MMP-2 and MMP-9 mRNA (16). Proteins in the MMP family are classified into three categories based on the degradation substrate: Collagenases (for example, MMP-1 and MMP-13), gelatinases (for example, MMP-2 and MMP-9), which act on denatured collagen and collagen types IV and V, and stromelysins (for example, MMP-3) (17). Therefore, miR-21 not only regulates the number of NPCs, but also regulates in the expression of MMP.

As another potentially important miRNA, miR-10b has not only been found to be expressed in a variety of tissue types, but also has several functions (38), and its abnormal expression is closely associated with the occurrence of malignant tumors dominated by uncontrollable cell proliferation (39). Compared with the NP tissue of patients with idiopathic scoliosis, miR-10b expression in degenerated NP tissues is significantly increased and is closely associated with the degree of disc degeneration (40). *In vitro*, miR-10b overexpression was shown to stimulate NPC proliferation and inhibit the translation of the homeobox D10 (HOXD10) gene, whereas restored HOXD10 expression reversed the pro-mitotic effect of miR-10b (40). miR-10b-mediated downregulation of HOXD10 expression resulted in increased Ras homolog gene family member C (RhoC) expression and Akt phosphorylation. By downregulating RhoC or inhibiting Akt, the effects of miR-10b on NPC proliferation were eliminated (40). This suggests that abnormal upregulation of miR-10b in IDD may result in abnormal proliferation of NPCs by targeting HOXD10 to inhibit the RhoC/Akt pathway.

miR-96 was found to be upregulated in human degenerated NP tissue and was positively correlated with the degree of IDD (41). Overexpression of miR-96 may promote NPC proliferation by targeting AT-rich interaction domain 2 to activate the Akt signaling pathway (41). miR-665 is similar to miR-9 in that its expression increases with the aggravation of disc degeneration. The increased expression of miR-665 not only promotes NPC proliferation, but also reduces aggrecan and type II collagen expression and increases MMP-3 and MMP-13 expression by inhibiting the expression of growth differentiation factor 5 in NPCs (42). miR-125b-1-3p regulates the cell cycle proteins cyclin D1 and B1 by targeting teashirt zinc finger homeobox 3, which may be involved in the regulation of NPC proliferation (43). In addition, miR-184 was elevated in degenerative NP tissues and promoted abnormal proliferation of NPCs via the growth arrest-specific 1/Akt pathway (44). The abnormal proliferation of NPCs is one of the early changes observed in IDD, and some miRNAs are involved in this important pathological change (Table I). Therefore, these miRNAs are expected to represent targets for the early detection and prevention of IDD.

## 4. miRNAs and NPC apoptosis

Apoptosis, or programmed cell death, is an important factor in IDD. NPCs are the main source of ECM in IVD tissue. When NPCs become apoptotic, the amount of ECM is reduced, and the water in the IVD tissue cannot be retained, resulting in the loss of biomechanical properties (45). NPC apoptosis is considered to be an important mechanism involved in IDD, and some miRNAs have been reported to be involved in the regulation of apoptosis in NPCs (Table II) (46-64).

A growing body of literature suggests that miR-494 plays an important role in the regulation of apoptosis in NPCs. It has been found that miR-494 is upregulated in degenerated human IVD tissue, and inhibition of miR-494 may protect NPCs from TNF-α-induced apoptosis by targeting JunD and cytochrome *c* (46). In addition, miR-494 promotes the expression of ECM resolution factors, such as MMPs and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), by directly targeting sex-determining region Y-box (SOX)9 and reducing the expression of type II collagen and aggrecan, which mediates the apoptosis of degenerated human NPCs (47). Some scholars have observed elevated miR-494 expression levels in IDD rats, whereas miR-494 inhibitors reduced caspase-3 and Bax expression, and increased neurooncological ventral antigen 1 (NOVA1) and Bcl-2 expression (48). NOVA1 was identified as a target gene of miR-494 by a dual-luciferase reporter assay (48). miR-494 is widely involved in the apoptosis of NPCs and plays an important role in the progression of IDD. Therefore, it may represent a promising potential target for IDD treatment.

Recent research has found decreased expression of miR-129-5p in human IDD, whereas NPCs treated with

Table I. miRNAs are involved in NPC proliferation in IDD.

| Author (year) | miRNA | Experimental model | Expression | Target | (Refs.) |
|---|---|---|---|---|---|
| Liu *et al*, 2014 | miR-21 | hIDD tissue, hNPC | Up | PTEN | (14) |
| Sheng *et al*, 2018 | miR-21 | Rat IDD model | Up | HIF-1α/VEGF | (15) |
| Chen *et al*, 2016 | miR-21 | hNP tissue, hNPC | Up | PDCD4 | (16) |
| Yu *et al*, 2013 | miR-10b | hIDD tissue, hNPC | Up | HOXD10 | (40) |
| Tao *et al*, 2017 | miR-96 | hIDD tissue, hNPC | Up | ARID2/AKT | (41) |
| Tan *et al*, 2018 | miR-665 | hNP tissue, hNPC | Up | GDF5 | (42) |
| Li *et al*, 2017 | miR-184 | hNP tissue, hNPC | Up | GAS1 | (44) |

miR/miRNA, microRNA; IDD, intervertebral disk degeneration; h, human; NP, nucleus pulposus; NPC, NP cell; PDCD4, programmed cell death 4; HOXD10, homeobox D10; ARID2, AT-rich interaction domain 2; GDF, growth differentiation factor; GAS1, growth arrest-specific 1.

Table II. miRNAs involved in NPC apoptosis in IDD.

| Author (year) | miRNA | Experimental model | Expression | Target | (Refs.) |
|---|---|---|---|---|---|
| Wang *et al*, 2015 | miR-494 | hNPC | Up | JunD | (46) |
| Kang *et al*, 2017 | miR-494 | hNP tissue, hNPC | Up | SOX9 | (47) |
| Li *et al*, 2018 | miR-494 | hNPC, rat IDD model | Up | NOVA1 | (48) |
| Yang and Sun, 2019 | miR-129-5p | hIDD tissue, NPC | Down | BMP2 | (49) |
| Sun *et al*, 2019 | miR-499a-5p | hNPC, hNP tissue | Down | SOX4 | (50) |
| Yang *et al*, 2019 | miR-143-5p | Rat IDD model, rat NPC | Up | eEF2 | (51) |
| Liu *et al*, 2017 | miR-125a | hNP tissue, hNPC | Down | BAK1 | (52) |
| Wang *et al*, 2011 | miR-155 | hNP tissue, hNPC | Down | FADD/caspase-3 | (28) |
| Zhao *et al*, 2017 | miR-143 | hNP tissue, hNPC | Up | BCL2 | (54) |
| Lv *et al*, 2018 | miR-30d | hNP tissue, hNPC | Up | SOX9 | (55) |
| Liu *et al*, 2013 | miR-27a | hNP tissue, hNPC | Up | PI3K | (56) |
| Wang *et al*, 2016 | miR-138-5p | hNP tissue, hNPC | Down | SIRT1 | (57) |
| Sun *et al*, 2018 | miR-532 | hNPC, h-blood | Up | Bcl-9 | (58) |
| Wang *et al*, 2019 | miR-573 | hNP tissue, hNPC | Down | Bax | (59) |
| Liu *et al*, 2016 | miR-4458 | hNP tissue, hNPC | Up | IGF1 | (60) |
| Zhang *et al*, 2016 | miR-210 | hNP tissue hNPC | Down | HOXA9 | (61) |
| Cai *et al*, 2017 | miR-15a | hNP tissue, hNPC | Up | MAP3K9 | (62) |
| Liu *et al*, 2019 | miR-222-3p | hNPC | Up | CDKN1B | (63) |
| Wang *et al*, 2018 | miR-199 | hNPC | Down | MAP3K5 | (64) |

miR/miRNA, microRNA; h, human; NP, nucleus pulposus; NPC, NP cell; IDD, intervertebral disk degeneration; SOX, sex-determining region Y-box; HOXA9, homeobox A9; IGF1, insulin-like growth factor 1; CDKN1B, cyclin-dependent kinase inhibitor 1B; NOVA1, neurooncological ventral antigen 1; eEF2, eukaryotic elongation factor 2; BAK1, Bcl2 antagonist/killer 1; SIRT, sirtuin; FADD, Fas-associated protein with death domain.

miR-129-5p mimics or bone morphogenic protein 2 (BMP-2) siRNA exhibited improved survival and inhibition of apoptosis (49). Therefore, the abnormal expression of miR-129-5p may serve a role in IDD by targeting BMP-2. Similar to miR-129-5p, miR-499a-5p was also found to be significantly downregulated in human degenerated NP tissue. miR-499a-5p knockout promoted NPC apoptosis, stimulated caspase activation, enhanced MMP-3 and MMP-13 expression, and decreased aggrecan and type II collagen expression (50). In addition, overexpression of miR-499a-5p alleviated the apoptosis of TNF-α-treated NPCs and the imbalance of ECM anabolism and catabolism; however, the abnormal expression of SOX4 weakened the negative effect of miR-499a-5p on NPC apoptosis and the positive effect on ECM synthesis (50). This indicates that the effects of miR-499a-5p may be mediated by targeting SOX4. NPCs are the main functional cells of the IVD. The ECM produced by NPCs is the structural basis of the biomechanical properties of the IVD.

Consequently, the abnormal proliferation of NPCs is an early pathological change of IDD (44) and may be helpful for the early identification of IDD. Whether the function of NPCs after early abnormal proliferation is the same as those of the parental NPCs needs further study. The ECM in IVD tissue is mainly derived from NPCs, and the apoptosis of NPCs will

Table III. miRNAs involved in inflammation in IDD.

| Author (year) | miRNA | Experimental model | Expression | Target | (Refs.) |
|---|---|---|---|---|---|
| Gu *et al*, 2015 | miR-146a | IL1-NPC (bovine tail) | / | IL-1 | (69) |
| Lv *et al*, 2017 | miR-146a | PBMCs/LPS-hNPC | Down | IL-1b/IL-6, TNF-α | (70) |
| Chen *et al*, 2019 | miR-194-5p | hFOB, hAFC, hNPC, hIDD tissue | Down | CUL4A/B | (71) |
| Qin *et al*, 2019 | miR-149 | LPS-NPC (rat) | Down | MyD88 | (72) |
| Zhang *et al*, 2019 | miR-222 | hNPC, hIDD tissue | Up | TIMP3 | (73) |
| Sun *et al*, 2018 | miR-155 | Rat NPC, hNPC, hNP tissue | / | TCF7L2 | (74) |
| Shen *et al*, 2019 | miR-625-5p | hAFC, hNPC, hIDD tissue | Up | COL1A1 | (75) |
| Lu *et al*, 2018 | miR-589-3p | hNPC | Up | SMAD4 | (76) |
| Zhang *et al*, 2018 | miR-140-5p | hNPC, hIDD tissue | Down | TLR4 | (77) |
| Dong *et al*, 2019 | miR-640 | hNPC, hAFC, hIDD tissue | Up | LRP1 | (78) |
| Li *et al*, 2018 | miR-148a | hNPC, hIDD tissue | Down | p-p38 | (79) |
| Wang *et al*, 2018 | miR-223 | Rat NP tissues, rat NPC | Down | Irak1 | (80) |
| Kong *et al*, 2018 | miR-194 | LPS-NPC (rat) | Down | TRAF6 | (81) |
| Zhou *et al*, 2019 | miR-155 | Rat NPC, C57BL/6 mouse | / | C/EBPβ | (82) |
| Cao and Chen, 2017 | miR-27a | hIDD tissue, hNPC | Up | IL-1β/IL-6/TNF-α | (83) |

miR/miRNA, microRNA; NP, nucleus pulposus; PBMCs, peripheral blood mononuclear cells; LPS, lipopolysaccharide; h, human; FOB, osteoblast cell; AFC, annuls fibrosus cell; NPC, NP cell; IDD, intervertebral disc degeneration; CUL4, cullin family gene 4; MyD88, myeloid differentiation factor 88; TIMP, tissue inhibitor of metalloproteinase; COL1A1, collagen, type I, alpha 1; TLR, toll-like receptor; TCF7L2, transcription factor 7 like 2; LRP1, LDL receptor-related protein-1; Irak1, IL-1 receptor-associated kinase 1; TRAF6, TNFR-associated factor 6; C/EBP, CCAAT/enhancer binding protein.

accelerate the degeneration of the IVD. Further understanding the association between miRNAs and NPC apoptosis in the process of IDD may provide new approaches to delaying or reversing IDD.

## 5. miRNAs and inflammation

Inflammation is considered to be an important mechanism in the IDD process. The concentrations of nitric oxide, prostaglandin E2, IL-1β, IL-6 and TNF-α in degenerated IVDs were found to be higher compared with those in normal IVDs (64-66). Furthermore, IL-1β and TNF-α mediate catabolism and anti-anabolism within the NP, which are largely involved in the establishment and progression of IDD (67,68). In recent years, numerous studies (69-83) have revealed that miRNAs may be involved in IDD through the regulation of inflammation (Table III).

miR-146a has been reported to inhibit the mRNA expression of IL-1-mediated inflammatory genes and catabolic proteinases, as well as the protein level of IL-1-mediated MMPs and aggrecanases (69). In 2017, Lv *et al* (70) demonstrated that the expression of miR-146a in peripheral blood mononuclear cells of patients with IDD was significantly downregulated. In addition, they found that overexpression of miR-146a could significantly downregulate the levels of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in lipopolysaccharide-stimulated NPCs, and confirmed that these effects depend on the TNF receptor associated-associated factor 6/NF-κB pathway.

miR-194-5p was also found to be significantly downregulated in patients with IDD by miRNA-based microarray analysis (71). Inhibition/overexpression of miR-194-5p led to inhibition/overexpression of cullin family (CUL) gene 4A (CUL4A) and CUL4B (71). Furthermore, IL-6 and TNF-α inhibitors in NPCs and AF cells reduced the expression of CUL4A and CUL4B (71). Similar to miR-194-5p, miR-149 was significantly reduced in lipopolysaccharide-induced NPCs (71). Overexpression of miR-149 reversed the expression of aggrecan and collagen II, and alleviated the increase in MMP-3, ADAMTS4 and inflammatory cytokines by targeting myeloid differentiation factor 88 (72). *In vitro*, miR-222 mimics/inhibitors were able to promote/inhibit NPC apoptosis, respectively (74). Moreover, transfection of miR-222 mimics/inhibitors could significantly increase/reduce the production of TNF-α, IL-1β, and IL-6 and inhibit/enhance the expression of collagen II and aggrecan, respectively (73).

Therefore, miRNAs directly or indirectly affect the ECM, NPCs and AF cells of the IVD through the regulation of inflammatory factors, subsequently influencing the process of IDD. Taking specific miRNAs as the entry point to control the progression of inflammation in the process of IDD may be an important approach to delaying the progression of IDD.

## 6. miRNAs and ECM remodeling

In human IVDs, the ECM is mainly composed of proteoglycans and type II collagen, which not only retain water, but also help maintain osmotic pressure, thus conferring unique biomechanical properties (84). The balance of ECM catabolism and anabolism is the basis of the biomechanical function of the IVD. An important feature of IDD is that ECM catabolism is greater than its anabolism (85). Recently, a number of studies demonstrated that miRNAs may be involved in the regulation of the ECM in IDD by regulating key molecules (for example,

Table IV. miRNAs involved in ECM remodeling in IDD.

| Author (year) | miRNA | Experimental model | Expression | Target | (Refs.) |
|---|---|---|---|---|---|
| Ye *et al*, 2016 | miR-155 | hNPC | Down | ERK1/2 | (86) |
| Zhang *et al*, 2017 | miR-155 | hIDD | Down | MMP-16 | (87) |
| Jing and Jiang, 2015 | miR-93 | hNP tissue | Down | MMP-3 | (88) |
| Hua *et al*, 2017 | miR-127-5p | hNP tissue | Down | MMP-13 | (89) |
| Yang *et al*, 2019 | miR-483-3p | hNPC | Up | GSK3B | (91) |
| | miR-23c | | Down | CTNNB1 | |
| Ji *et al*, 2016 | miR-98 | hNP tissue | Down | MMP-2 | (25) |
| Zhang *et al*, 2018 | miR-3150a-3p | hNPC | Up | ACAN | (92) |
| Yan *et al*, 2015 | miR-100 | hIDD | Up | MMP-13 | (93) |
| Zhou *et al*, 2017 | miR-146a | hNP tissue | Up | / | (94) |
| Liu *et al*, 2016 | miR-7 | hNP tissue | Up | GDF5 | (95) |
| Kang *et al*, 2017 | miR-15b | hNP tissue | Up | SMAD3 | (96) |
| Liu *et al*, 2017 | miR-132 | hNP tissue | Up | GDF5 | (97) |
| Wang *et al*, 2018 | miR-21 | hNPC | Up | PTEN | (98) |
| Yang *et al*, 2019 | miR-146 | Rat NPC | / | Notch1 | (99) |
| Shi *et al*, 2019 | miR-202-3p | hNPC | Down | MMP-1 | (100) |
| Wang *et al*, 2017 | miR-210 | hNP tissues | Up | ATG7 | (101) |
| Chai *et al*, 2019 | miR-486-5p | LPS-stimulated NPC | Down | FOXO1 | (102) |
| Wang *et al*, 2019 | miR-154 | hNPC | Up | FGF14 | (103) |

h, human; NP, nucleus pulposus; NPC, NP cell; IDD, intervertebral disc degeneration; ECM, extracellular matrix; PBMCs, peripheral blood mononuclear cells; LPS, lipopolysaccharide; FOB, osteoblast cell; AFC, annuls fibrosus cell; GSK, glycogen synthase kinase; GDF, growth differentiation factor; CTNNB1, β-catenin; ACAN, aggrecan; ATG7, autophagy-related gene 7; FOXO1, forkhead box O1; FGF, fibroblast growth factor.

MMPs, collagen II and ADAMTS) that affect anabolic and catabolic processes (25,86-103) (Table IV).

In degenerated disc tissue, inhibition of miR-155 was shown to reduce the expression of collagen II and glycosaminoglycans by targeting ERK1/2 (86). A previous study using an IDD mouse model demonstrated that upregulation of miR-155 upregulated the expression of aggrecan and collagen type II, and down-regulated MMP-16 (87), suggesting that miR-155 serves an important regulatory role in ECM anabolism and catabolism.

MMPs are classified into three categories based on the degradation substrate: Collagenases, gelatinases, or stromelysins (37). MMPs are key molecules regulating ECM catabolism. Several miRNAs targeting the MMP family participate in the regulation of ECM metabolism (18-20,88,89). These findings indicate that the involvement of miRNAs in ECM catabolism may be closely associated with the regulation of MMP expression. The effects of MMPs on ECM catabolism and anabolism are important. Therefore, by regulating MMPs, these miRNAs may represent important biological molecules in the alleviation, or even the reversal of ECM loss.

Reversing or alleviating ECM loss is crucial for IDD. Aucubin, a compound found in traditional Chinese medicines, was reported to play a key role in the regulation of the ECM (90). In IDD, the increased release of pro-inflammatory factors by NPCs can cause the degradation of the ECM. However, aucubin can alleviate the degradation of ECM mediated by IL-1β or TNF-α by regulating the miR-140/cAMP responsive element binding protein 1 axis (90). In addition, Bu Shen Hu Xue Fang (BSHXF), a traditional Chinese medicine, is composed of six herbs (*Cortex Eucommiae ulmoides*, *Fructus Psoraleae*, *Achyranthes bidentata*, *Salvia miltiorrhiza*, *Radix clematidis* and *Chaenomeles speciosa*). Through the regulation of the Wnt signaling pathway by miR-483-3p and miR-23c, BSHXF was shown to affect ECM synthesis and NPC proliferation (91). Thus, traditional Chinese medicine may also be of value in the study of IDD.

## 7. miRNAs and CEP changes

The CEP is located between the vertebral endplate and the NP, and is mainly composed of hyaline cartilage cells and chondrocytes, and the ECM they produce. CEP degradation is accompanied by a loss of nutrients in the ECM, which is a major cause for the development of IDD (104). CEP serves as a mechanical shock absorber and is also an important channel for the free transmission of nutrients and metabolites between the avascular NP and the vertebral body (105). It also serves as a barrier and part of the body's defense against toxic and harmful substances, such as inflammatory factors, MMPs and immune molecules, entering the NP (106,107). The occurrence of IDD may be associated with CEP degeneration, dysfunction, and calcification (108,109). Some miRNAs (110-114) have been found to be involved in the changes of the CEP in the progress of IDD (Table V).

Chondrocytes are an important part of the CEP. However, it was demonstrated that upregulation of miR-34a expression

Table V. miRNAs involved in CEP changes in IDD.

| Author (year) | miRNA | Experimental model | Expression | Target | (Refs.) |
|---|---|---|---|---|---|
| Chen *et al*, 2015 | miR-34a | Chondrocytes (hIDD) | Up | Bcl-2 | (110) |
| Zhan *et al*, 2018 | miR-625 | hIDD | Down | Fas | (111) |
| Sheng *et al*, 2018 | miR-221 | Cells (hIDD CEP) | Up | ERα | (112) |
| Zheng *et al*, 2019 | miR-365 | Chondrocytes (hIDD CEP) | Down | HDAC4 | (114) |
| Liu *et al*, 2016 | miR-20a | Cells (hCEP) | Up | ANKH | (116) |

h, human; IDD, intervertebral disc degeneration; CEP, cartilaginous endplate; HDAC, histone deacetylase; ANKH, ankylosis protein homolog; ER, estrogen receptor.

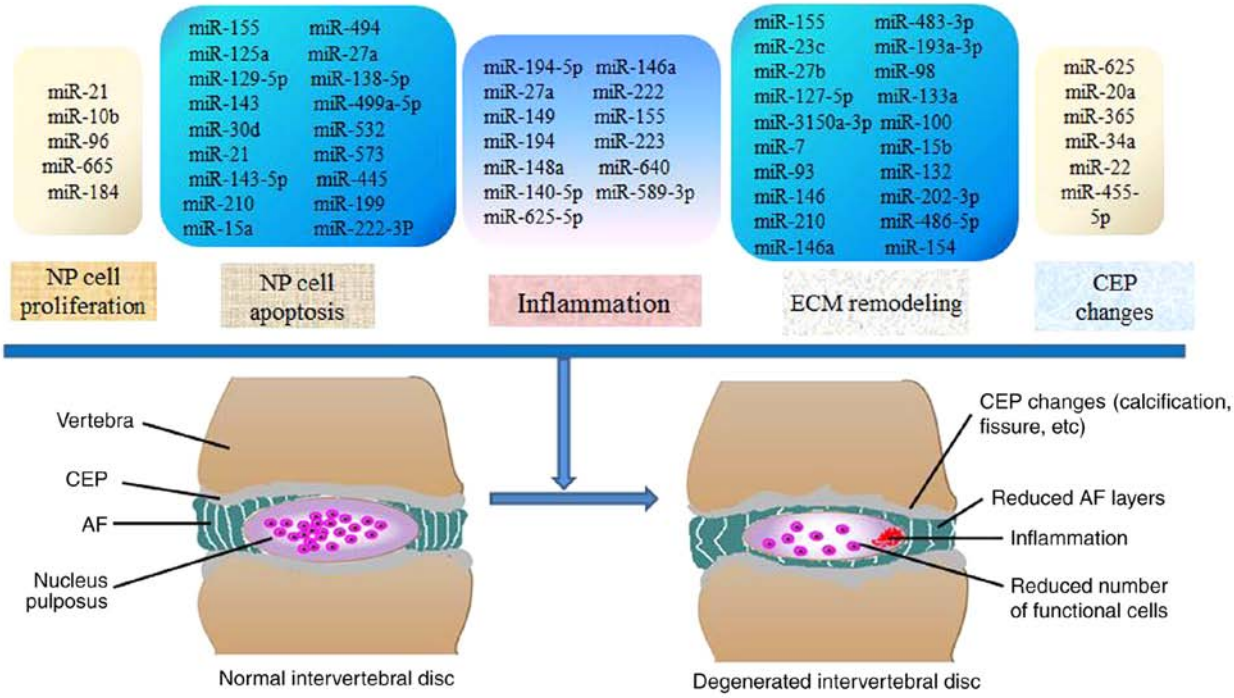


Figure 1. miRNAs promote IDD progression through multiple pathological processes, including NP cell proliferation and apoptosis, inflammation, ECM remodeling and CEP changes. IDD, intervertebral disk degeneration; NP, nucleus pulposus; ECM, extracellular matrix; CEP, cartilaginous endplate; AF, annulus fibrosus; miR/miRNA, microRNA.

in human degenerated chondrocytes caused Fas-mediated CEP chondrocyte apoptosis (110). Similar to miR-34a, down-regulation of miR-625 also caused Fas-mediated cervical CEP chondrocyte apoptosis (111). These findings may indicate that the effects of miRNAs on chondrocyte apoptosis in the CEP may be mediated by Fas. Further research may elucidate the role of miRNAs in the apoptosis of CEP chondrocytes.

Interestingly, estrogen (17β-estradiol, E2) could inhibit apoptosis of CEP cells and restore cell viability and cell cycle progression in the G0/G1 phase (112). The luciferase assay demonstrated that estrogen receptor α (ERα) was a target of miR-221 (112). This indicates that miR-221 may affect the protective effect of estrogen on degenerating CEP cells through targeting ERα. However, there are few studies on estrogen in IDD, and its specific effect and mechanism require further research.

The CEP is an important load-bearing structure, and long-term mechanical loads are considered to be one of the causes of IDD (113). miR-365 is a mechanically sensitive miRNA, which directly targets histone deacetylase 4 to regulate the degeneration of human chondrocytes (114). In addition, the TGF-β/SMAD signaling pathway inhibits the intermittent cyclic mechanical tension-mediated degeneration of CEP chondrocytes by regulating the miR-455-5p/Runt-related transcription factor 2 (Runx2) axis (115). In addition, it was previously demonstrated that miR-20a/ankylosis protein homolog regulates stiff matrix-promoted CEP calcification (116). Chondrocyte degeneration and calcification affect the function of the CEP, which is an important factor in CEP degeneration.

## 8. miRNAs and AF

The AF consists of bundles of fibers arranged in a crisscross pattern and is an important structure surrounding the NP. AF cells may be a source of pluripotent stem cells with the

potential to differentiate into adipocytes, chondrocytes, neurons, osteoblasts and endothelial cells (117). The normal AF and degenerated AF cells were found to undergo osteogenic differentiation, as shown by mineralization of cultured cells and increased mRNA expression of BMP2, Runx2, alkaline phosphatase and osteocalcin (118). However, the osteogenic differentiation potential of degenerated AF cells is higher than that of normal AF cells, which may be associated with the regulation of the BMP/SMAD pathway by miR-221 (118). AF is an important structure of the IVD. However, the association between pathological changes in the AF and miRNAs in IDD remains elusive.

## 9. miRNAs and IDD diagnosis

Early treatment intervention usually improves the outcome of most diseases; however, early diagnosis is a prerequisite for early intervention. There are currently no early diagnostic methods for IDD, and imaging findings are often lacking. Therefore, it is necessary to explore and develop laboratory diagnostic methods for early diagnosis of IDD. The expression level of miR-26a-5p in the serum of mice with IVD degeneration was found to be consistently higher compared with that of young pre-injury samples or a normal control group without IVD degeneration (119). This indicates that miR-26a-5p is a potential molecule for IDD diagnosis. In addition, the expression of miR-146a in peripheral blood mononuclear cells from patients with IDD was significantly lower compared with that of healthy controls (70). Some scholars recently demonstrated that several miRNAs (for example, miR-199a-5p, miR-574-3p, miR-551a and miR-640) may be candidate markers for predicting IDD (120). The clinical correlation between these miRNAs and IDD suggests that miRNAs may be useful as early diagnostic markers of IDD.

## 10. Conclusion

IDD is currently a common disease. However, there is yet no optimal treatment for IDD, and the main reason is that its pathogenesis is unknown. miRNAs participate in the various pathological processes implicated in IDD (Fig. 1). Significant progress has been made in the study of miRNAs affecting the development of IDD, revealing the association between genetic susceptibility and exposure to risk factors, and improving our understanding of the pathogenesis of IDD. Taken together, the findings of the currently available studies highlight miRNAs as a promising research direction for IDD. Further study on the association between miRNAs and IDD may reveal new diagnostic markers and therapeutic targets for IDD.

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## Availability of data and materials

Not applicable.

## Authors' contributions

QY and SG conceived this review article. FY, JW, ZC, YY and WZ searched the literature and collected the articles/published data, for inclusion and interpretation in this review. All the authors were involved in the writing of the manuscript. All the authors have read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

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## Competing interests

The authors declare that they have no competing interests.

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