Emerging evidence on noncoding-RNA regulatory machinery in intervertebral disc degeneration: a narrative review

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Abstract

Intervertebral disc degeneration (IDD) is the most common cause of low-back pain. Accumulating evidence indicates that the expression profiling of noncoding RNAs (ncRNAs), including microRNAs (miRNAs), circular RNAs (circRNAs), and long noncoding RNAs (lncRNAs), are different between intervertebral disc tissues obtained from healthy individuals and patients with IDD. However, the roles of ncRNAs in IDD are still unclear until now. In this review, we summarize the studies concerning ncRNA interactions and regulatory functions in IDD. Apoptosis, aberrant proliferation, extracellular matrix degradation, and inflammatory abnormality are tetrad fundamental pathologic phenotypes in IDD. We demonstrated that ncRNAs are playing vital roles in apoptosis, proliferation, ECM degeneration, and inflammation process of IDD. The ncRNAs participate in underlying mechanisms of IDD in different ways. MiRNAs downregulate target genes' expression by directly binding to the 3'-untranslated region of mRNAs. CircRNAs and IncRNAs act as sponges or competing endogenous RNAs by competitively binding to miRNAs and regulating the expression of mRNAs. The lncRNAs, circRNAs, miRNAs, and mRNAs widely crosstalk and form complex regulatory networks in the degenerative processes. The current review presents novel insights into the pathogenesis of IDD and potentially sheds light on the therapeutics in the future.

Keywords: Apoptosis, Cell proliferation, Extracellular matrix degeneration, Intervertebral disc degeneration, Inflammation, Noncoding-RNA, Nucleus pulposus cell

Background

Intervertebral disc degeneration (IDD) is the most common cause of low-back pain, which affects over 70% of people at some points of their whole lifetime. However, due to the poor understandings of the pathogenesis of the disorder, few treatment regimens have been put forward, and none of the current clinical interventions for IDD has been confirmed as efficient and radical treatment modalities. Therefore, an in-depth investigation of the regulatory machinery of IDD is urgently needed in the present.

Intervertebral disc (IVD) can be divided into three morphologically distinct regions, i.e., the sandwiched central nucleus pulposus (NP), peripheral annulus fibrosus (AF), and cranial or caudal cartilaginous endplate (CEP) (Fig. 1). During the process of IDD, the apoptosis of IVD cells is abnormally increased with the cells aberrantly clustering, dysregulation of extracellular matrix (ECM) proteins (abnormally synthesized and/or degraded), and excessive expression of inflammatory factors which accelerate the formation of inflammatory microenvironment/niche and eventually violate the adjacent IVD cells. These pathophysiological
Emerging evidence reveals that genetic and environmental factors are both influencing factors of IDD, whereas genetic factors seem to be the outweighed one. Notably, a multitude of genetic factors, implicating in the underlying regulatory mechanisms, are dysregulated in IDD, especially the noncoding RNAs (ncRNAs) [6, 11, 12]. NcRNAs consist of a large family of RNAs without coding function and outcome as cellular effectors, i.e., proteins. So far, the identified ncRNAs in homo sapiens include miRNAs, circRNAs, lncRNAs, and emerging small RNAs. The expression profiling of ncRNAs of IDD samples is significantly different from those from healthy ones, reflected by differentially expressed levels and types of ncRNAs unraveled by microarray and/or sequencing analyses. It is suggested that ncRNAs are playing vital roles in apoptosis, proliferation, ECM degeneration, and inflammation process of IDD [12–17]. Owing to that, we established the coding-noncoding SuperSeries Datasets as GSE67567 in human IDD, including lncRNAs, mRNAs, and circRNAs, and miRNAs.
datasets as GSE19943, GSE63492, GSE56081, and GSE67566, as well as studies from other investigators (Table 1). Given the scarcity of studies summarizing the research progress of ncRNAs in IDD, we designed and conducted a review across the published papers [13, 17, 18, 20–25]. In the current work, the state-of-art research

| Data accession number | Types of RNA profiling | Platform | BioProject | Samples | Control set | Publication year | Contributors |
|-----------------------|------------------------|----------|------------|----------|-------------|-----------------|--------------|
| GSE19943              | miRNAs                | GPL9946  | PRJNA120173| GSM498350 | 3 control (scoliosis) vs. 3 degenerative nucleus pulposus (NP) cell samples, extracted from NP tissue without cultures | 2011          | Wang et al. [18] |
| GSE45856              | miRNAs                | GPL11434 | PRJNA196506| GSM1116694| 3 control (traumatic normal) vs. 3 degenerative IVD tissues using TRIspin method | 2013          | Zhao et al. [19] |
| GSE56081              | miRNAs                | GPL15314 | PRJNA242356| GSM134764 | 5 control (cadaveric normal) vs. 5 degenerative NP tissues using TRIspin method | 2014          | Wan et al. [13] |

| GSE19943              | LncRNAs               | GPL19449 | PRJNA268036| GSM1551024 | 5 control (cadaveric normal) vs. 5 degenerative NP tissues using TRIspin method | 2016          | Lan et al. [20] |
| GSE67566              | circRNAs              | GPL19978 | PRJNA280274| GSM1649704 | 5 control (cadaveric normal) vs. 5 degenerative NP tissues using TRIspin method | 2016          | Lan et al. [20] |
| GSE67567              | Noncoding RNA SuperSeries | GPL15314 | PRJNA280271| In combination | 5 control (cadaveric normal) vs. 5 degenerative NP tissues | 2016          | Lan et al. [20] |
| GSE153761             | LncRNAs, miRNAs and circRNAs | GPL22120 | PRJNA643990| GSM4653870 | 3 control (traumatic normal) vs. 3 degenerative cartilage endplate of cervical disc | 2020          | Yuan et al.      |
advance and therapeutic potentials concerning the regulatory roles of miRNAs/circRNAs/lncRNAs in degenerated discs of human or animal models were summarized and discussed (Fig. 1).

The regulatory mechanism of miRNAs in IDD
The expression profile and molecular mechanisms of miRNAs in IDD
Accumulating evidence indicates that the miRNA expression profile in IDD cases is significantly different from those in the controls. In 2011, we presented the first line of evidence on miRNA expression profiling in IDD, using scoliosis NP tissues as control. Twenty-nine differentially expressed miRNAs were identified, with 6 upregulated and 23 downregulated [18]. Thereafter, the emerging molecules as miRNAs catch the attention of global researchers, manifesting as an increasing number of published studies. Subsequently, Zhao et al. compared the expression profile of miRNAs between IDD and spinal cord injury patients in 2014. Twenty-six miRNAs were downregulated in the IDD group, while 25 upregulated [19]. Further investigation revealed these dysregulated miRNAs controlled several signaling pathways, which are pivotal in the pathogenesis of IDD, such as Wnt [19, 26, 27], phosphoinositide 3-kinase/Akt (PI3K/Akt) [19, 28], and mitogen-activated protein kinase (MAPK) [19, 29], etc. Consistently, Hu et al. demonstrated that among the 253 miRNAs detected both in IDD and scoliosis samples, three were downregulated and six were upregulated in degenerative samples. The downstream targets were predicted to be genes or proteins associated with degeneration, such as drosophila mothers against decapentaplegic protein family member 4 (SMAD4), which play important roles in cell-cycle-related pathways [30].

Complementary base sequence endows miRNAs the ability to bind the 3′untranslated region (3′UTR) of particular mRNA. The binding of miRNAs and mRNAs results in a decreased expression of the target proteins [31, 32], while most of them are hub proteins, which play a crucial role in essential pathways associated with degeneration. Thus, miRNAs indirectly control the pathological processes in disc degeneration. The IDD-related miRNAs are presented in Table 2.

In summary, 49 miRNAs were reported with a relationship to IDD, among the total number of 38,589 miRNAs of Homo sapiens, according to miRBase Release 22.1 (http://www.mirbase.org/). Whereas studies have been focused on intra-cellular miRNAs, cell-free miRNAs emerge as potential novel biomarkers for a variety of human diseases. Recently, eRNA Atlas has been proposed across human biofluids, which is also essential in the regulation of IDD [86].

The roles of miRNAs in IVD cell apoptosis
Accumulating evidence shows that several miRNAs function as inducers or inhibitors in the apoptosis of IVD cells via specific target genes or pathways [87]. For instance, downregulated miR-155 was suggested triggering the Fas-mediated apoptosis by disinhibiting FADD and CASP-3 in NP cells [18]. Similarly, the expression of miR-21 [36], miR-499a-5p [40], miR-486-5p [46], miR-125a [51], miR-145 [56], and miR-573 [57] are decreased in IDD, which act as apoptosis inhibitors via binding to the 3′UTRs of miRNAs of PTEN, SOX4, FOXO1, TP53INP1, ADAM17, and Bax, respectively. In contrast to these findings, miRNAs such as miR-27a [39], miR-494 [41, 42], miR-30d [43], miR-222-3p [44], miR-15a [45], miR-143 [49], miR-532 [50], miR-138-5p [55] in NP cells, miR-106a-5p [8] in AF cells, and miR-34a [7] and miR-221 [52] in CEP cells, display potential pro-apoptotic effects in IDD, via inhibiting the expression of downstream hub proteins in several pathways. Apart from the aforementioned mechanisms, miRNAs, i.e., miR-153-3p, participating in the autophagy, also contributes to the disc degeneration eventually [58].

In summary, there are eight miRNAs acting as inhibitors of apoptosis in IDD, whereas eleven miRNAs act as promoters of apoptosis.

The roles of miRNAs in IVD cell proliferation
Cell number in healthy human IVDs is limited and sparsely distributed. However, the cells were reported to proliferate into clusters in IDD [88]. In this complex pathophysiological process, multiple miRNAs acting as vital indirect regulators in IVD cell proliferation can be employed as biomarkers. For example, in NP cells, the aberrant overexpression of miR-21 increases the proliferation level of degenerated NP cells by downregulating PDCD4 and PTEN. Thus, the disinhibition effect increased the phosphorylation level of c-Jun and AKT proteins, which could induce cell proliferation. Liu et al. found that miR-21 knockdown reversed cell proliferation, while Ly294002, an AKT inhibitor, reversing the effect induced by miR-21. These results indicate that miR-21 is a potential biomarker and therapeutic target of IDD [21, 37].

Besides, overexpression of miR-10b [59], miR-96 [60], miR-184 [61], miR-2355-5p [62], and miR-665 [65] could also promote the proliferation of degenerated NP cells via targeting PTEN/PDCD4, HOXD10, ARID2, GASI, ERFFI1, and GDF5, while upregulation of miR-222-3p [44], miR-15a [45], and miR-125b-1-3p [64] had an opposite effect by inhibiting the expression of CDKN1B, MAP3K9, and TSHZ3. These downstream genes regulate NP cell proliferation by controlling crucial pathways, such as RhoC-Akt pathway [59], PTEN/AKT pathway [21, 37], ARID2/AKT signaling [60], and activating/deactivating molecular molecules like AKT.
| MiRNA     | Expression | Target(s)                                                                 | Functions                                  | Publication year | References |
|-----------|------------|---------------------------------------------------------------------------|--------------------------------------------|------------------|------------|
| MiR-155  | ↓          | FADD, caspase-3, ERK1/2, TCF7L2, MMP-16                                    | ↓ NP cell apoptosis, ECM degradation        | 2011             | [18]       |
|           |            |                                                                           |                                             | 2016–2018        | [33–35]    |
| MiR-21   | ↑          | PTEN                                                                      | ↓ NP cell apoptosis, ↑ NP cell proliferation | 2018             | [36]       |
|           | ↑          | PTEN, PDCD4                                                                | ↑ NP cell proliferation, ↓ ECM degradation  | 2014, 2016       | [21, 37]   |
|           |            |                                                                           |                                             | 2018             | [38]       |
| MiR-27a  | ↑          | PI3K                                                                       | ↓ NP cell apoptosis                        | 2013             | [39]       |
| MiR-499a-5p | ↓       | SOX4                                                                       | ↓ NP cell apoptosis, ↑ ECM degradation      | 2019             | [40]       |
|           |            |                                                                           |                                             | 2019             | [40]       |
| MiR-494  | ↑          | SOX9, JunD, SOX9                                                           | ↑ NP cell apoptosis, ↑ ECM degradation      | 2015, 2017       | [41, 42]   |
|           |            |                                                                           |                                             | 2017             | [41]       |
| MiR-30d  | ↑          | SOX9                                                                       | ↑ NP cell apoptosis, ↓ ECM degradation      | 2019             | [43]       |
|           |            |                                                                           |                                             | 2019             | [43]       |
| MiR-222-3p | ↑        | CDKN1B                                                                    | ↑ NP cell apoptosis, ↑ ECM degradation      | 2019             | [44]       |
|           |            |                                                                           |                                             | 2019             | [44]       |
| MiR-15a  | ↑          | MAP3K9                                                                     | ↑ NP cell apoptosis, ↓ ECM degradation      | 2017             | [45]       |
|           |            |                                                                           |                                             | 2017             | [45]       |
| MiR-486-5p | ↓         | FOXO1                                                                      | ↓ NP cell apoptosis, ↑ ECM degradation, ↑ inflammation | 2019             | [46]       |
|           |            |                                                                           |                                             | 2019             | [46]       |
| MiR-200c | ↑          | XIAP                                                                       | ↑ NP cell apoptosis, ↑ ECM degradation      | 2018             | [23]       |
|           |            |                                                                           |                                             | 2018             | [23]       |
| MiR-328-5p | ↑         | ERBB2                                                                      | ↑ NP cell apoptosis                         | 2018             | [47]       |
| MiR-34a  | ↑          | GDF5, Bcl-2                                                                | ↑ ECM degradation                           | 2016             | [48]       |
|           |            |                                                                           |                                             | 2015             | [7]        |
| MiR-143  | ↑          | Bcl-2                                                                       | ↑ NP cell apoptosis                         | 2017             | [49]       |
| MiR-532  | ↑          | Bcl-9                                                                       | ↑ NP cell apoptosis                         | 2018             | [50]       |
| MiR-125a | ↓          | TPS3IP1                                                                    | ↓ NP cell apoptosis                         | 2016             | [51]       |
| MiR-221  | ↑          | ERα, FOXO3, TRPS1, BMP-Smad pathway                                         | ↑ CEP cell apoptosis, ↑ ECM degradation     | 2018             | [52]       |
|           |            |                                                                           |                                             | 2018             | [52]       |
|           |            |                                                                           |                                             | 2018             | [52]       |
|           |            |                                                                           |                                             | 2018             | [52]       |
|           |            |                                                                           |                                             | 2016             | [54]       |
| MiR-138-5p | ↑         | SIRT1                                                                      | ↑ NP cell apoptosis                         | 2016             | [55]       |
| MiR-145  | ↓          | ADAM17                                                                     | ↓ NP cell apoptosis, ECM degradation         | 2019             | [56]       |
|           |            |                                                                           |                                             | 2019             | [56]       |
| MiR-573  | ↓          | Bax                                                                         | ↓ NP cell apoptosis                         | 2019             | [57]       |
| MiR-153-3p | ↓        | ATG5                                                                        | ↓ NP cell autophagy                         | 2019             | [58]       |
| MiR-106a-5p | ↑       | ATG7                                                                       | ↑ AF cell apoptosis, ECM degradation         | 2019             | [8]        |
|           |            |                                                                           |                                             | 2019             | [8]        |
| MiR-10b  | ↑          | HOXD10                                                                      | ↑ NP cell proliferation                     | 2013             | [59]       |
| MiR-96   | ↑          | ARID2                                                                       | ↑ NP cell proliferation                     | 2017             | [60]       |
| MiR-184  | ↑          | GAS1                                                                       | ↑ NP cell proliferation                     | 2017             | [61]       |
| MiR-2355-5p | ↑      | ERFF11                                                                      | ↑ NP cell proliferation, ↑ inflammation     | 2019             | [62]       |
|           |            |                                                                           |                                             | 2019             | [62]       |
| MiR-365  | ↓          | HDAC4                                                                       | ↑ CEP cell proliferation                    | 2019             | [63]       |
| MiR-125b-1-3p | ↑    | TSH23                                                                       | ↓ NP cell proliferation                     | 2018             | [64]       |
| MiR-665  | ↑          | GDF5                                                                        | ↑ NP cell proliferation, ↑ ECM degradation  | 2018             | [65]       |
|           |            |                                                                           |                                             | 2018             | [65]       |
| MiR-7    | ↑          | GDF5                                                                        | ↑ ECM degradation                            | 2016             | [66]       |
| MiR-132  | ↑          | GDF5                                                                        | ↑ ECM degradation                            | 2017             | [67]       |
| MiR-15b  | ↑          | SMAD3                                                                       | ↑ ECM degradation                            | 2017             | [68]       |
Among them, miR-222-3p promotes the proliferation of IVD cells and accelerates the apoptosis and ECM degradation via the same pathway [44]. In accordance with this, miR-15a [45], miR-106a-5p [8] and miR-17-3p [72] have a similar effect, which limits their application as therapeutic targets.

In addition to NP cells, miR-106a-5p [8] in AF cells and miR-365 [63] in CEP cells are also associated with cell proliferation, by inhibiting the proliferation level via ATG7 and increasing proliferation via HDAC4, respectively.

Collectively, there were 12 miRNAs involved in IVD cell proliferation, with eight miRNAs promoting proliferation and four miRNAs inhibiting proliferation.

### The roles of miRNAs in ECM degradation and inflammation

Generally, IVD cells play an essential role in secreting ECM components like collagens and proteoglycans to maintain IVD’s structural stability and resist mechanical loads [89, 90]. However, in IDD, the unbalance between synthesis and degradation of ECM makes the IVD unrenewable and degenerative, especially in NP tissues [91]. MiRNAs modulate the degradation of ECM by regulating the expression of essential enzymes such as matrix metalloproteinases (MMPs) or cytokines such as interleukins.

It is reported that inhibition of miR-665 [65], miR-7 [66], miR-132 [67], and miR-34a [48] effectively attenuate ECM degradation in degenerative NP tissues by directly upregulating the expression of growth differentiation factor-5 (GDF5), which can inhibit the expression of ECM catabolic factors, such as MMP and ADAMTS4, and upregulating the production of anabolic proteins, such as type II collagen and aggrecan.

A series of miRNA, i.e., miR-202-3p [71], miR-17-3p [72], miR-93 [73], miR-133a [74], miR-27b [75], miR-127-5p [76], miR-193a-3p [77], and miR-98 [33] are significantly downregulated in degenerative NP tissues, with their expression levels reversely correlated with the grade of IDD, which induce type II collagen synthesis via directly suppressing the expression levels of MMP1, MMP2, MMP3, MMP9, MMP13, MMP14, and MMP16, respectively, whereas overexpression of miRNAs mentioned above can stop and reverse the degradative process, indicating that they are potential biomarkers and therapeutic targets of IDD.

In addition, two different protective mechanisms of miR-155 have been clarified in ECM degradation. Ye et al. have shown that the knockdown of miR-155 results

| MiRNA | Expression | Target(s) | Functions | Publication year | References |
|-------|------------|-----------|-----------|-----------------|------------|
| MiR-20a | ↑ | ANKH | ↑ CEP chondrocyte calcification | 2016 | [69] |
| MiR-377 | ↑ | ADAMTS5 | ↑ ECM degradation | 2013 | [70] |
| MiR-202-3p | ↓ | MMP1 | ↓ ECM degradation | 2019 | [71] |
| MiR-17-3p | ↓ | MMP2 | ↓ ECM degradation | 2018 | [72] |
| MiR-93 | ↓ | MMP3 | ↓ ECM degradation | 2015 | [73] |
| MiR-133a | ↓ | MMP9 | ↓ ECM degradation | 2016 | [74] |
| MiR-27b | ↓ | MMP13 | ↓ ECM degradation | 2016 | [75] |
| MiR-127-5p | ↓ | MMP13 | ↓ ECM degradation | 2017 | [76] |
| MiR-193a-3p | ↓ | MMP14 | ↓ ECM degradation | 2016 | [77] |
| MiR-98 | ↓ | IL-6 | ↓ ECM degradation | 2016 | [78] |
| MiR-100 | ↑ | FGFR3 | ↑ ECM degradation | 2015 | [79] |
| MiR-146a | Not clear** | TRAF6 | ↑ ECM degradation | 2015,2017 | [22, 80] |
| MiR-210 | ↑ | ATG7 | ↑ ECM degradation | 2017 | [81] |
| MiR-194 | ↑ | CHSY1/2/3 | ↑ ECM degradation | 2017 | [82] |
| MiR-515 | ↑ | CHSY1/2/3 | ↑ ECM degradation | 2017 | [82] |
| MiR-3150a-3p | ↑ | ACAN | ↑ ECM degradation | 2018 | [83] |
| MiR-640 | ↑ | LRP1, β-catenin, EP300 | ↑ inflammation | 2019 | [84] |
| MiR-140-5p | ↓ | TLR4 | ↓ inflammation | 2018 | [85] |

* Decrease in apoptotic NP cells. ** It is reported that miR-146a is significantly downregulated in the PBMCs of IDD patients, but its expression in NP cells is unclear [80]

The expression, targets, and functions of miRNAs related to IDD were displayed in Table 2. "↑" represents upregulation, while "↓" represents downregulation.

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in decreased expressions of collagen II and glycosaminoglycan by increasing the expression of ERK1/2 [34]. Sun et al. have reported that an essential transcription factor, TCF7L2, which acts as an activator in the process of chondrocyte matrix degradation through \(p65/NF-kB\) signaling, was repressed by miR-155 [35].

Wang et al. discovered that miR-21 is upregulated in IDD tissues and positively correlated with the degradation grade, which indicates miR-21 cannot only inhibit NP cell apoptosis and promote proliferation as mentioned above, but also promote ECM degradation through repressing the \(PTEN/AKT/mTOR\) signaling pathway [38]. SRY-related high-mobility group box (SOX)-4 and SOX9 are respectively targeting molecules of miR-499a-5p [40], miR-494 [41], and miR30d [43], by repressing the apoptosis of NP cells and ECM degradation.

As well, a number of miRNAs can affect the process of ECM degradation, including miR-222-3p [44], miR-486-5p [46], miR-221 [52, 53], miR-145 [56], and miR-98 [78] in NP tissues, miR-221 [54] in AF tissues, and miR-20a [69] in CEP tissues. The expression, targets, and functions of these miRNAs are listed in Table 2.

Apart from apoptosis, proliferation, and ECM degradation, inflammation responses and inflammatory cytokines are also regarded as crucial factors in the pathogenesis of IDD [92]. miRNAs associated with the production of inflammation cytokines, such as miR-486-5p [46], miR-221 [52], miR-2355-5p [62], miR-146a [22, 80], miR-640 [84], and miR-140-5p [85] are also listed in Table 2 and can also be used as therapeutic targets of IDD. In general, there are six reported miRNAs pertaining to inflammation during IDD in various subparts of the IVDs via a multitude of targeting genes, affecting a variety of inflammatory cytokines. Three deregulated miRNAs (miR-140-5p targeting \(TLR4\) [85], miR-486-5p targeting \(FOXO1\) [46] and miR-146a targeting \(TRAF6\) [22, 80]; all studying in NP cells) are associated with decreased levels of inflammation, whereas three miRNAs (miR-221 targeting \(ERa\) in CEP cells [52], miR-640 targeting \(LRP1\), \(\beta\)-catenin and \(EP300\) in NP and AF cells [84], and miR-2355-5p targeting \(ERFF1\) in NP cells [62]) are linked with increased levels of inflammation during IDD.

The regulatory mechanism of circRNAs in IDD

The profile and mechanism of circRNAs in IDD

CircRNAs are a group of single-stranded RNAs with loop structures, which act as competing endogenous RNAs (ceRNAs) and restore the functions of specific genes by sponging miRNAs [17, 93]. A specific miRNA could be sponged by various distinct circRNAs, forming a circRNA-miRNA-mRNA interaction network [20, 47, 72]. Thus, circRNAs seems like a critical regulator in gene expression.

We presented the first line of evidence of circRNAs expression profiling in human IDD In 2016. We found 636 differentially expressed circRNAs in human lumbar IVDs, with 354 upregulated and 282 downregulated [20]. Zou and colleagues indicated that many genes regulated by circRNAs are playing crucial roles in the pathogenesis of IDD, via over 15 signaling pathways, such as Wnt and integrin signaling pathways. Pairs of host genes and circRNA can be divided into four categories according to their profile: circRNA and its host genes downregulated, circRNA and its host genes upregulated, circRNA downregulated and its host genes upregulated, and circRNA upregulated and its host genes downregulated [93].

Several experiments were conducted to investigate the differences between the profile of circRNAs in degenerative IVDs and that in normal IVDs. Wang et al. have provided another line of evidence that 72 circRNAs were upregulated by more than two-fold in degenerative NP tissues [94]. Following this, another team identified there were 7294 circRNAs aberrantly expressed (3724 upregulated, 3570 downregulated, fold change > 2) in degenerative NP cells [17]. Recently, Li et al. reviewed the results from related publications from 2016 to 2019 and confirmed that the profile in IDD patients is different from that in the control group, with the number of upregulated circRNAs ranging from 51 to 3724, and the number of downregulated circRNAs ranging from 21 to 3570 [15].

As the dynamic development of miRBase reflecting novel findings in miRNAs, multiple circRNA databases have been proposed with changing numbers and updated findings as well. So far, there are hundreds of human circRNAs reported (148 in chondrocytes and 104 in osteocytes) [95].

The roles of circRNAs in IDD

Specific circRNA can indirectly regulate apoptosis, proliferation, and ECM degradation by modulating the level of functional miRNA, contributing to the disc degeneration. Specifically, CircVMA21 [23], Circ-GRB10 [47], and CircRNA\(_{104670}\) [72] are involved in apoptosis regulation. CircRNA\(_{104670}\) [72] and CircSEMA4B [96] are related to NP cell proliferation. CircVMA21 [23], Circ-4099 [94], CircSEMA4B [96], and CircRNA\(_{104670}\) [72] are associated with ECM degradation.

As shown in Table 3, miR-200c accelerates the apoptosis of NP cell and ECM degradation via inhibiting \(\text{XIAP}\), whereas CircVMA21 alleviates the negative effect of sponging miR-200c. However, in degenerative IVD tissues, the expression of CircVMA21 is repressed, resulting in aberrant higher level of miR-200c and IDD [23]. Circ-GRB10 is downregulated in degenerative NP tissues. Transient overexpression of GRB10 could attenuate the apoptosis of NP cells by sequestering miR-
328-5p and leading to the activation of genes associated with proliferation via the ErbB pathway [47]. Besides, overexpression of circSEMA4B could inhibit NP cells from proliferation and ECM degradation induced by IL-1β via indirectly rescuing SFRP1 or GSK-3β in Wnt signaling from miR-431 [96]. Song et al. found that upregulated CircRNA_104670 accelerates apoptosis and inhibits cell proliferation and collagen II synthesis in NP cells via circRNA_104670/miR-17-3p/MMP2 network [72]. In contrast, upregulated Circ-4099 acts as a protective factor by disinhibiting the expression of collagen II and aggrecan and downregulating the expression of the pro-inflammatory factors such as TNF-α, and PGE2 by sponging miR-616-5p. The expression data, targeted miRNAs, and functions of circRNA in IDD are listed in Table 3.

The regulatory mechanism of IncRNAs in IDD

The profile and mechanism of IncRNAs in IDD

IncRNAs are a group of ncRNAs with more than 200 nucleotides. IncRNAs take the role of ceRNAs (as circRNAs) or small interfering RNA (siRNAs) and participate in the IncRNA/circRNA/miRNA/mRNA network as transcriptional regulators [97]. They regulate gene expression or control the signaling pathways by competitively sponging and inactivating specific miRNAs [12, 13]. Some IncRNAs even regulate the activity or stability of proteins by directly interacting with them [98, 99]. Investigations indicate that IncRNAs exert their regulatory function in various ways (i.e., reducing the methylation level of the promoter region may accelerate the expression of specific IncRNAs in IVD cells [100]). Therefore, the aberrant expression of IncRNAs will cause the degeneration of IVD cells and result in the development of IDD.

Ample evidence indicates that the profile of IncRNAs in degenerative IVDs is totally different from those in normal IVDs. In 2014, we reported the first expression profiling of IncRNAs in human IDD by using the same human lumbar IVD samples as circRNAs. One hundred sixteen IncRNAs (with 67 upregulated and 49 downregulated) and 260 mRNAs were differentially expressed in degenerative samples with an absolute fold change greater than ten [13]. Among the deregulated IncRNAs in IDD, HOTAIR (NR_003716) is the top downregulated IncRNAs (fold change, 148.53; P < 0.001) [13]. Later, Zhao et al. reported that 1530 of 1854 differential expressed IncRNAs might have 6386 potential target genes, whereas Han et al. reported 632 IncRNAs are differentially expressed in IDD tissues among 40,716 detected IncRNAs [101, 102]. Li and colleagues reviewed the articles related to expression profiles of IncRNAs and summarized the number of differentially expressed IncRNAs. The number of upregulated IncRNAs is ranging from 67 to 2234, while the downregulated ones ranging from 49 to 938 [97]. These results indicate that IncRNAs could modulate the destiny of NP cells in IDD and be transformed into screening biomarkers or therapeutic targets.

The roles of IncRNAs in IDD

The roles of IncRNAs in IDD can be divided into four main categories according to their functions (apoptosis, cell proliferation, ECM degradation, inflammation) as well. A specific IncRNA can have two or more functions simultaneously.

Chen et al. found that overexpression of TUG1 in degenerative NP samples accelerates cell apoptosis, via up-regulating the levels of Bax&caspase-3 (the latter are pro-apoptotic factors) in Wnt1/β-catenin pathway and downregulating the levels of Bel-2, an anti-apoptotic factor. In addition, the increased level of TUG1 also deteriorates the degradation of ECM by breaking the expression balance in the ECM-degrading and anti-ECM-degrading genes [24]. Both GAS5 and IncPoE are overexpressed in degenerative IVD samples, displaying similar roles in apoptosis. While GAS5 increases the apoptosis by binding to miR-155, IncPoE negatively regulates PoE [100, 103].

Emerging evidence suggests that autophagy is an essential process in IDD and has a close relationship with
apoptosis. Zhang and colleagues reported that overexpression of HOTAIR accelerates NP cell apoptosis via stimulating cell autophagy [104]. On the contrary, Shao et al. indicated that downregulated HOTAIR expression inhibits cell apoptosis via the Notch signaling pathway by sponging miR-34a-5p. In other words, the overexpression of HOTAIR reduces NP cell apoptosis [105].

Aberrant cell proliferation is another core pathogenesis in IDD. SNHG1 promotes NP cell proliferation via sponging miR-326, and downregulated miR-326 disinhibits NP cell proliferation by inactivating PCNA and cyclin D1 expression. Similarly, RP11-296A18.3/miR-138/HIF1A, MRMP/miR-206/PCNA, H19/miR-22/LEF1/Wnt/β-catenin signaling, and HCG18/miR-146a-5p/TRAF6/NF-kB axis can also increase or decrease the level of proliferation, respectively [10, 25, 106–108]. Targeting extracellular signal-regulated kinase (Erk) and miR-146a-5p/TRAF6/NF-kB axis, respectively, IncRNA FAF1 and HCG18 modulate the ratio of synthesis-phase cells among all the cells in NP tissue [109].

H19, Linc00958, and SLC20A1 have been reported to upregulate ECM degradation via sponging miRNAs [107, 109, 110]. It is noteworthy that H19 plays a role as a competitor to LEF1 for binding miR-22, regulating Wnt/β-catenin pathway [107]. Linc00958 and NEAT1 exert their function by increasing the expression of MMPs via upregulating SMAD and inhibiting the synthesis of aggrecan and collagen-II in the ERK/MAPK pathway, respectively [109, 111]. Wei et al. demonstrated that decreased FAM83H-AS1 in IDD results in ECM degeneration, by targeting Notch1 and Hes1 [112]. While Linc–ADAMTS5, interacting with splicing factor proline/glutamine-rich (SFPQ), which induces the down expression of ADAMTS5, alleviates the ECM deterioration process [113].

Inflammatory cytokines and inflammatory cytokine-related lncRNAs are also involved in IDD. Several members of the interleukin family, such as IL-1 and IL-6, were widely noted as pro-inflammatory factors, giving rise to the degeneration of ECM and apoptosis of IVD cells. In vitro studies showed that overexpression of MALAT1 attenuates IL-1 and IL-6 induced inflammation by sponging miR-503, displaying a protective effect on IVD cell [114]. Besides, ZFAS1 is linked with inflammatory cytokine levels in IDD. Since the positive correlation between the intensity of inflammatory and severity of degeneration, ZFAS1 is regarded as a sensitive predictor of IDD [115].

LncRNAs related to the modulation and prediction of IDD are listed in Table 4.

Conclusion

In recent years, a large number of investigations have depicted a bright future for ncRNAs, which play roles as delicate regulators in the pathogenesis of IDD. The IncRNA/circRNA/miRNA/mRNA networks and the widespread crosstalks between the RNAs provide us another way to recognize and understand the pathogenesis of IDD [19]. A number of aberrantly expressed RNAs have been regarded as early diagnostic biomarkers or useful therapeutic targets. Moreover, novel materials and technologies, such as injectable hydrogel or nanoparticle which is loadable for small RNAs [117], genetic technologies and stem cell-based therapies [118, 119], are developing rapidly, making it possible to interfere the RNA expression inside IVD cells.

The rapid development of high throughput biotechnological tools greatly facilitates the studies for ncRNAs in IDD. The most common biotechnological approaches are microarray analysis for specific ncRNAs and/or sequencing technologies. Following successful RNA isolation and quality control, ncRNA expression in IDD can be detected via developed microarray chips with known covered ncRNA numbers and types according to corresponding ncRNA database versions. Alternatively, ncRNA expression in IDD can be studied using next-generation sequencing platforms following reverse transcription to cDNA. Thereafter, sequencing data can be mapped to human genomic version (the updated version as GRCh38) and annotated into various subtypes of ncRNAs, with the pros of uncovering novel ncRNAs and cons as introducing errors/mutations during reverse transcription. A combined exploration of both biotechnologies might overcome the cons and improve the studies of ncRNAs in IDD. Novel sequencing technologies are needed for direct sequencing of RNAs and omitting the reverse transcription step. In addition, there are triple common tools/techniques for ncRNA studies following screening. First, RT-PCR tool aims for the detection of expression levels of ncRNAs. Second, bioinformatics and online software tools apply for ncRNA function, target, and interaction predictions. Third, in vitro modulation (upregulation and repression) designates for target and function validations.

However, we are still facing with lots of challenges. Lack of knowledge about the overall view of the ncRNA networks makes it challenging to identify the key nodes to interfere with. The roles of tRNAs and emerging small RNAs, i.e., small nucleolar RNAs (snRNAs) and PIWI-interacting RNAs (piRNAs), which may be equally
important in IDD, remain unclear and deserve thorough studies. Stem cells, such as mesenchymal stem cells (MSCs), have already been used in IVD degeneration therapies for assisting tissue regeneration and exosome secretion, which contains miRNAs to improve microenvironment. However, the inflammatory milieu of IVDs is tough for MSCs to survive in degenerative IVD tissues [120, 121]. Thus, improvement in tissue engineering techniques is urgently needed in seed cell implanting [122]. Future studies should keep focusing on the molecular mechanisms of crosstalk among ncRNAs, especially novel snoRNAs, piRNAs, and tRNAs, and seek feasible ways in seed cell implantation, nanoparticles containing RNA molecules or engineered tissues to interfere the hub nodes in the regulatory network. With the issues solved, research advances in the regulatory machinery of ncRNAs will provide the medical community with a brighter future for IDD therapies.

**Table 4** Experimentally verified IncRNAs associated with IDD

| LncRNA | Expression | Target(s) | Functions | Publication year | References |
|--------|------------|-----------|-----------|------------------|------------|
| TUG1   | ↑          | Wnt1/β-catenin, Bax & caspase-3 | ↑ NP cell apoptosis; ↑ ECM degradation | 2017 [24] |            |
|        |            |           |           |                  |            |
| GAS5   | ↑          | miR-155   | ↑ NP cell apoptosis; ↑ ECM proliferation | 2017 [24] |            |
|        |            |           |           |                  |            |
| LncPolE| ↑          | PolE      | ↑ NP cell apoptosis | 2019 [103] |            |
|        |            |           |           |                  |            |
| HOTAIR | ↑          | AMPK/mTOR/ULK1 | ↑ NP cell apoptosis; ↓ miR-34a-5p | 2020 [104] |            |
|        | ↓          | miR-503   | ↓ NP cell apoptosis; ↓ Inflammation | 2017 [114] |            |
|        |            |           |           |                  |            |
| MALAT1 | ↓          | miR-503   | ↓ NP cell apoptosis; ↓ ECM proliferation; ↓ ECM degradation | 2017 [114] |            |
|        |            |           |           |                  |            |
| LINC00641| ↑       | miR-153-3p | ↑ NP cell autophagy | 2019 [58]  |            |
|        |            |           |           |                  |            |
| SNHG1  | ↑          | miR-326   | ↑ NP cell proliferation | 2018 [106] |            |
|        |            |           |           |                  |            |
| RP11-296A18.3| ↑   | miR-138   | ↑ NP cell proliferation; ↑ ECM synthesis | 2017 [10]  |            |
|        |            |           |           |                  |            |
| H19    | ↑          | miR-22    | ↑ NP cell proliferation; ↑ ECM degradation | 2018 [107] |            |
|        |            |           |           |                  |            |
| FAF1   | ↑          | Erk       | ↑ NP cell proliferation | 2018 [116] |            |
|        |            |           |           |                  |            |
| FAM83H-AS1| ↑     | Notch1    | ↑ NP cell growth; ↑ ECM degradation | 2019 [112] |            |
|        |            |           |           |                  |            |
| HCG18  | ↑          | miR-146a-5p | ↑ NP cell apoptosis; ↑ ECM degradation | 2017 [108] |            |
|        |            |           |           |                  |            |
| Linc00958| ↑      | miR-203   | ↑ NP cell proliferation; ↑ ECM degradation | 2019 [109] |            |
|        |            |           |           |                  |            |
| RMRF   | ↑          | miR-206   | ↑ NP cell proliferation; ↑ ECM degradation | 2018 [25]  |            |
|        |            |           |           |                  |            |
| NEAT1  | ↑          | ERK1/2, MAPK | ↑ ECM degradation | 2018 [111] |            |
|        |            |           |           |                  |            |
| SLC20A1| ↑          | miR-31-5p | ↑ ECM degradation | 2017 [113] |            |
|        |            |           |           |                  |            |
| Linc-ADAMTSS| ↑      | SFQ       | ↑ ECM degradation | 2017 [114] |            |
|        |            |           |           |                  |            |
| ZFAS1  | ↑          |          | Associate with inflammation level | 2019 [115] |            |

The expression, targets, and functions of IncRNAs related to IDD were displayed in Table 4. “↑” represents upregulation, while “↓” represents downregulation.

**Abbreviations**

3'UTR: 3' Untranslated region; ADAMTS: A disintegrin and metalloprotease with thrombospondin motifs; AF: Annulus fibrosus; CDKN1B: Cyclin-dependent kinase inhibitor 1B; CEP: Cartilaginous endplate; CellRAs: Competing endogenous RNAs; CircRNA: Circular RNA; Col I: Type I collagen; Col II: Type II collagen; ECM: Extracellular matrix; ER: Estradiol receptor; FADD: Fas-associated death domain-containing protein; FGFR3: Fibroblast growth factor receptor-3; HOXD10: Homeobox D10; IDD: Intervertebral disc degeneration; IL-1β: Interleukin-1β; IVD: Intervertebral disc; IncRNA: Long noncoding RNA; MAPK: Mitogen-activated protein kinase; miRNAs: MicroRNAs; MMP: Matrix metalloproteinases; NcRNAs: Noncoding RNAs; NP: Nucleus pulposus; PDCD4: Programmed cell death 4; PI3K: Phosphoinositide 3-kinase; PTEN: Phosphate and tension homology deleted on chromosome ten; SAA1: Serum amyloid A1; SMAD4: Mothers against decapentaplegic protein family member 4; SOX: SRY-related high-mobility group box; TGF-β: Transforming growth factor-β; TNF-α: Tumor necrosis factor-α; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand

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**Authors’ contributions**

HQW conceived the study. HYG, MKG, and FS investigated and retrieved the published papers. HYG and MKG analyzed data. HYG wrote the original draft.
ZYW review and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
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Competing interests
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References
1. Frymoyer JW, Cats-Baril WL. An overview of the incidences and costs of low back pain. Orthop Clin North Am. 1991;22(2):263–71.
2. Nakamura M, Nishiwaki Y, Ushida T, Toyama Y. Prevalence and characteristics of chronic musculoskeletal pain in Japan. J Orthop Sci. 2011; 16(4):424–32.
3. Nakamura M, Toyama Y, Nishiwaki Y, Ushida T. Prevalence and characteristics of chronic musculoskeletal pain in Japan: a second survey of people with or without chronic pain. J Orthop Sci. 2014;19(2):339–50.
4. Konovalov NA, Nazarenko AG, Astyutin DS, Zelenkov PV, Onoprienko RA, Korolishin VA, Cherkev IU, Martynova MA, Zakirov BA, Timonin SY, et al. Modern treatments for degenerative disc diseases of the lumbar spine. A literature review. Zh Vopr Neirokhir Im N N Burdenko. 2016;80(4):102–8.
5. Lee CK, Langrana NA. A review of spinal fusion for degenerative disc disease: need for alternative treatment approach of disc arthroplasty? Spine J. 2004;4(6 Suppl):S173–65.
6. Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? Spine (Phila Pa 1976). 2006;31(18):2151–61.
7. Chen H, Wang J, Hu B, Wu X, Chen Y, Li R, Yuan W. MiR-34a promotes Fas-mediated cartilage endplate chondrocyte apoptosis by targeting Bcl-2. Mol Cell Biochem. 2015;406(1–2):21–30.
8. Hai B, Ma Y, Pan X, Yong L, Liang C, He G, Yang C, Zhu B, Liu X. Melatonin benefits to the growth of human annulus fibrosus cells through inhibiting miR-106a-5p/ATG7 signaling pathway. Clin Interv Aging. 2019;14:621–30.
9. Vergroesen PP, Kingma I, Emanuel KS, Hoogendoorn RJ, Welting TJ, van Royen BJ, van Dieën JH, Smit TH. Mechanics and biology in intervertebral disc degeneration: a vicious circle. Osteoarthr Cartil. 2013;21(7):1057–70.
10. Wang X, Lv G, Li J, Wang B, Zhang Q, Lu C. LncRNA-RP11-296A18.3/miR-138/HIF1A pathway regulates the proliferation ECM synthesis of human nucleus pulposus cells (HNPCs). J Cell Biochem. 2017;118(12):4862–71.
11. Mayer JE, Iatridis JC, Chan D, Quek SA, Gottesman O, Hecht AC. Genetic polymorphisms associated with intervertebral disc degeneration. Spine J. 2013;13(3):299–317.
12. Zhu J, Zhang X, Gao W, Hu H, Wang X, Hao D. IncRNA/circRNA/miRNAs/celRNA network in lumbar intervertebral disc degeneration. Mol Med Rep. 2019;20(2):3166–74.
13. Wan ZY, Song F, Sun Z, Chen YF, Zhang WL, Samartzis D, Ma CJ, Che L, Liu X, Ali MA, et al. Aberrantly expressed long noncoding RNAs in human intervertebral disc degeneration: a microarray related study. Arthritis Res Ther. 2014;16(5):465.
14. Chen Y, Ni H, Zhao Y, Chen K, Li M, Li C, Zhu X, Fu Q. Potential role of IncRNAs in contributing to pathogenesis of intervertebral disc degeneration based on microarray data. Med Sci Monit. 2015;21:1344–58.
15. Li Z, Chen X, Xu D, Li S, Chan MTW, Wu WWK. Circular RNAs in nucleus pulposus cell function and intervertebral disc degeneration. Cell Prog. 2019; 5(2):el21704.
16. Wang C, Wang WJ, Yan YG, Xiang YX, Zhang J, Tang ZH, Jiang ZS. MicroRNAs: new players in intervertebral disc degeneration. Chin Clin Acta. 2015;450:333–41.
17. Wang S, Sun J, Yang H, Zou W, Zheng B, Chen Y, Guo Y, Shi J. Profiling and bioinformatics analysis of differentially expressed circular RNAs in human intervertebral disc degeneration. Acta Biochim Biophys Sin (Shanghai). 2019;51(6):571–9.
18. Wang HQ, Yu XD, Liu ZH, Cheng X, Samartzis D, Jia LT, Wu SX, Huang J, Chen J, Luo ZJ. Deregulated miR-155 promotes Fas-mediated apoptosis in human intervertebral disc degeneration by targeting FADD and caspase-3. J Pathol. 2011;225(2):322–42.
19. Zhao B, Yu Q, Li H, Gao X, He X. Characterization of microRNA expression profiles in patients with intervertebral disc degeneration. Int J Mol Med. 2014;33(1):43–50.
20. Lan PH, Liu ZH, Pei YJ, Wu ZG, Yu Y, Yang YF, Liu X, Che L, Ma CJ, Xie YK, et al. Landscape of RNAs in human lumbar disc degeneration. Oncotarget. 2016;7(39):63166–76.
21. Chen B, Huang SG, Ju L, Li M, Nie FF, Zhang Y, Zhang YH, Chen X, Gao F. Effect of microRNA-21 on the proliferation of human degenerated nucleus pulposus by targeting programmed cell death 4. Braz J Med Biol Res. 2016;49(6):e5020.
22. Gu SX, Li X, Hamilton JL, Chee A, Kc R, Chen D, An HS, Kim JS, Oh CD, Ma YJ, et al. MicroRNA-146a reduces IL-1-dependent inflammatory responses in the intervertebral disc. Gene. 2015;555(2):80–7.
23. Cheng X, Zhang L, Zhang K, Zhang G, Hu Y, Sun X, Zhao C, Li H, Li YM. Zhao J. Circular RNA VMA21 protects against intervertebral disc degeneration through targeting miR-200C and X linked inhibitor-of-apoptosis protein. Ann Rheum Dis. 2018;77(5):770–9.
24. Chen J, Jia YS, Liu GZ, Sun Q, Zhang F, Ma S, Wang YJ. Role of LncRNA TUG1 in intervertebral disc degeneration and nucleus pulposus cells via regulating Wnt/beta-catenin signaling pathway. Biochem Biophys Res Commun. 2017;491(3):668–74.
25. Wang X, Peng L, Gong X, Zhang X, Sun R, Du J. LncRNA-RMRP promotes nucleus pulposus cell proliferation through regulating miR-206 expression. J Cell Mol Med. 2018;22(11):5468–76.
26. Hiyama A, Yokoyama K, Nakagawa T, Sakai D, Mochida J. A complex interaction between Wnt signaling and TNF-alpha in nucleus pulposus cells. Arthritis Res Ther. 2013;15(6):R189.
27. Hiyama A, Yokoyama K, Nakagawa T, Sakai D, Mochida J. Response to tumor necrosis factor-alpha mediated inflammation involving activation of prostaglandin E2 and Wnt signaling in nucleus pulposus cells. J Orthop Res. 2015;33(12):1756–68.
28. Zhang X, Hu Z, Hao J, Shen J. Low-intensity pulsed ultrasound stimulates the extracellular matrix synthesis of human degenerative nucleus pulposus cells via activating PI3K/Akt pathway. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2013;29(1):34–8.
29. Mao D, Zhang L. Leptin modulates the expression of catabolic genes in rat nucleus pulposus cells through the mitogen-activated protein kinase and Janus kinase 2/signal transducer and activator of transcription 3 pathways. Mol Med Rep. 2015;12(2):1761–8.
30. Hu P, Feng B, Wang G, Ning B, Jia T. Microarray based analysis of gene regulation by microRNA in intervertebral disc degeneration. Mol Med Rep. 2015;12(4):4925–30.
31. Guancial EA, Bellmunt J, Yeh S, Rosenberg JE, Berman DM. The evolving understanding of microRNA in bladder cancer. Urol Oncol. 2014;32(1):41 e31–40.
32. Yoshih H, Seki N, Ikaso T, Chiyomaru T, Nakagawa M, Enokida K. Alteration expression of microRNAs in bladder cancer. Nat Rev Urol. 2013; 10(7):396–404.

33. Zhang WL, Chen YF, Meng HZ, Du JJ, Luan GN, Wang HQ, Yang MW, Luo ZJ. Role of miR-155 in the regulation of MMP-16 expression in intervertebral disc degeneration. J Orthop Res. 2017;35(9):1523–34.

34. Ye D, Dai L, Yao Y, Qin S, Xie H, Wang W, Liang W. MiR-155 inhibits nucleus pulposus cells’ degeneration through targeting ERK 1/2. Dis Markers. 2016;2016:984270.

35. Sun J, Hong J, Sun S, Wang X, Peng Y, Zhou J, Huang Y, Li S, Chen W, Li C, et al. Transcription factor 7-like 2 controls matrix degradation through nuclear factor kappaB signaling and is repressed by miRNA-155 in nucleus pulposus cells. Biomed Pharmacother. 2018;108:64–55.

36. Cheng X, Zhang G, Zhang L, Hu Y, Zhang K, Sun X, Zhao C, Li H, Li YM, Zhao J. Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration. J Cell Mol Med. 2018;22(1):261–76.

37. Liu H, Huang X, Liu X, Xiao S, Zhang Y, Xiang T, Shen X, Wang G, Sheng B. miR-21 promotes human nucleus pulposus cell proliferation through PTEN/ AKT signaling. Int J Mol Sci. 2014;15(1):4007–18.

38. Wang W, Yang W, Ouyang ZH, Xue JB, Li XL, Zhang J, et al. Inhibition of microRNA-34a prevents IL-1beta-induced extracellular matrix degradation of degenerative human nucleus pulposus cells by targeting SOX9. Biomed Pharmacother. 2019;113:108652.

39. Liu G, Cao P, Chen H, Yuan W, Wang J, Tang X. MiR-27a regulates apoptosis in nucleus pulposus cells by targeting PTEN. PLoS One. 2013;8(9):e75251.

40. Sun JC, Zheng B, Sun RX, Meng YK, Wang SM, Yang HS, Chen Y, Shi JG, Guo YF. MiR-499a-5p suppresses apoptosis of human nucleus pulposus cells and degradation of their extracellular matrix by targeting SOX4. Biomed Pharmacother. 2019;113:108652.

41. Kang L, Yang C, Song Y, Zhao K, Liu W, Wu W, Wang K, Tu L, Li S, Yin H, et al. MicroRNA-494 promotes apoptosis and extracellular matrix degradation in degenerative human nucleus pulposus cells. Oncotarget. 2017;8(17):27868–81.

42. Wang T, Li P, Ma X, Tian P, Han C, Zang J, Kong J, Yan H. MicroRNA-494 inhibition protects nucleus pulposus cells from TNF-alpha-induced apoptosis by targeting JUNB. Biochemie. 2015;115:1–7.

43. Lv J, Li S, Tan W, Yang Y, Cheng Y, Xue R. Inhibition of microRNA-30d attenuates the apoptosis and extracellular matrix degradation of degenerative human nucleus pulposus cells by up-regulating SOX9. Chem Biol Interact. 2018;206X:89–97.

44. Liu J, Yu J, Jiang W, He M, Zhao J. Targeting of CDKN1B by miR-222-3p may attenuate the apoptosis of nucleus pulposus cells. Aging (Albany NY). 2018;10(7):396–9.

45. Guo W, Zhang B, Mu K, Feng SQ, Dong ZY, Ning GZ, Li HR, Liu S, Zhao L, et al. Circular RNA GRB10 as a competitive endogenous RNA regulating nuclear factor kappaB signaling and is repressed by microRNA-143 regulates apoptosis by targeting FOXO1 in intervertebral disc degeneration. J Cell Mol Med. 2017;21(11):4699–712.

46. Feng X, Yang T, Li Z, Yang Y, Song S, et al. MicroRNA-7 regulates IL-1beta-induced extracellular matrix degradation by targeting MAP3K9. Biomed Open Biol Interact. 2018;296:89.

47. Meng X, Zhu Y, Tao L, Zhao S, Qiu S. MicroRNA-125b-1-3p mediates proliferation and inflammation of nucleus pulposus cells by targeting GAS1. World Neurosurg. 2019;127:28–44. 10.1016/j.wneu.2019.03.012.

48. Guo Y, Tian L, Liu X, He Y, Chang S, Yen Y. EPDR11 inhibits proliferation and inflammation of nucleus pulposus and is negatively regulated by miR-235-5p in intervertebral disc degeneration. Spine (Phila Pa 1976). 2019;44(15):E873–81.

49. Zheng Q, Li XX, Xiao L, Shao S, Jiang H, Zhang XL, Sun LY, Xu HG. MicroRNA-365 functions as a mechanosensitive microRNA to inhibit end plate chondrocyte degeneration by targeting histone deacetylase 4. Bone. 2019;128:15052.

50. Meng X, Zhu Y, Tao L, Zhao S, Qiu S. MicroRNA-125b-1-3p mediates intervertebral disc degeneration in rats by targeting teashirt zinc finger homeobox 3. Exp Ther Med. 2018;15(3):2627–33.

51. Li W, Wang P, Zhang Z, Wang W, Liu Y, Qi Q. MiR-184 regulates proliferation in nucleus pulposus cells by targeting GAS1. World Neurosurg. 2017;99:710–5. e711.

52. Guo Y, Tian L, Liu X, He Y, Chang S, Yen Y. EPDR11 inhibits proliferation and inflammation of nucleus pulposus and is negatively regulated by miR-235-5p in intervertebral disc degeneration. Spine (Phila Pa 1976). 2019;44(15):E873–81.

53. Meng X, Zhu Y, Tao L, Zhao S, Qiu S. MicroRNA-125b-1-3p mediates proliferation and matrix degeneration of nucleus pulposus through targeting PTEN in intervertebral disc degeneration. J Cell Biochem. 2018;119(9):7218–25.

54. Liu W, Zhang Y, Li X, Fei Q, Gao Y, Wang K, Song Y, Duan Z, Yang S, et al. MicroRNA-7 regulates IL-1beta-induced extracellular matrix degradation by targeting PTEN in nucleus pulposus cells. Biomed Pharmacother. 2016;83:1414–21.

55. Liu W, Li X, Feng J, Kang L, Huang M, Wang K, Song Y, Li S, Wu X, Yang S, et al. MicroRNA-132 upregulation promotes matrix degradation in intervertebral disc degeneration. Exp Cell Res. 2017;359(1):39–49.

56. Kang L, Yang C, Yin H, Zhao K, Liu W, Hua W, Wang K, Song Y, Tu J, Li S, et al. MicroRNA-15b silencing inhibits IL-1beta-induced extracellular matrix degradation by targeting SADD3 in human nucleus pulposus cells. Biotechnol Lett. 2017;39(9):623–32.

57. Liu MH, Sun C, Yao Y, Fan X, Liu H, Cui YH, Bian XW, Huang B, Zhou Y. Matrix stiffness promotes cartilage endplate chondrocyte calcification in disc degeneration via miR-20a targeting ARH1 expression. Sci Rep. 2016;6:25401.

58. Tsitsouni E, Fedoridou C, Pneumisios SG, Tragas AA, Michalopoulos I, Mangoura D. PyKε activation stimulates ERK1/2, and regulates aggrecan, ADAMTS5, and miR377 gene expression in human nucleus pulposus cells. PLoS One. 2013;8(11):e82045.

59. Shi C, Liu W, Lin W, Cai Y, Zhang Y, Hu B, Gao R, Im HJ, Yuan W, Ye X, et al. MiR-202-3p regulates interleukin-1beta-induced expression of matrix metalloproteinase 1 in human nucleus pulposus. Gene. 2019;687:156–65.

60. Song J, Wang HL, Song RH, Ding ZW, Ma XS, Lu FX, Xiao L, Wang YW, Fei Z, Jia Y. CircularRNA_104670 plays a critical role in intervertebral disc degeneration by functioning as a ceRNA. Exp Mol Med. 2018;50(8):94.

61. Jing W, Jiang W. MicroRNA-93 regulates collagen loss by targeting MMP3 in human nucleus pulposus cells. Cell Mol Biol Lett. 2019;24(2):76.

62. Xu YQ, Zhang ZH, Zheng YF, Feng SQ. Dysregulated miR-133a mediates loss of type II collagen by directly targeting matrix metalloproteinase 9 (MMP9)
in human intervertebral disc degeneration. Spine (Phila Pa 1976). 2016; 41(2):E17–24.

75. Li HR, Cui Q, Dong ZY, Zhang JH, Li HQ, Zhao L. Downregulation of miR-27b is involved in loss of type II collagen by directly targeting matrix metalloproteinase-13 (MMP13) in human intervertebral disc degeneration. Spine (Phila Pa 1976). 2016;41(9):E16–23.

76. Hua WB, Wu XH, Zhang YK, Song Y, Tu J, Kang L, Zhao KC, Li S, Wang K, Liu W, et al. Dysregulated miR-127-5p contributes to type II collagen degradation by targeting matrix metalloproteinase-13 in human intervertebral disc degeneration. Biochimie. 2017;139:74–80.

77. Ji ML, Zhang XJ, Shi PL, Lu J, Wang SZ, Chang Q, Chen H, Wang C. Downregulation of microRNA-193a-3p is involved in intervertebral disc degeneration by targeting MMP14. J Mol Med (Berl). 2016;94(4):457–68.

78. Ji ML, Li J, Shi PL, Zhang XJ, Wang SZ, Chang Q, Chen H, Wang C. Dysregulated miR-98 contributes to extracellular matrix degradation by targeting IL-6/STAT3 signaling pathway in human intervertebral disc degeneration. J Bone Miner Res. 2016;31(4):900–9.

79. Yan N, Yu S, Zhang H, Hou T. Lumbar disc degeneration is facilitated by miR-100-mediated FGF3 suppression. Cell Physiol Biochem. 2015;36(6):2229–36.

80. Lv F, Huang Y, Lu W, Yang L, Li F, Fan J, Sun J. MicroRNA-146a ameliorates inflammation via TRAF6/NF-kappab pathway in intervertebral disc cells. Med Sci Monit. 2017;23:659–64.

81. Wang C, Zhang ZZ, Yang W, Ouyang ZH, Xue JB, Li XL, Zhang J, Chen JK, Yan YG, Wang WJ. MiR-210 facilitates ECM degradation by suppressing autophagy via silencing of ATG7 in human degenerated NP cells. Biomed Pharmacother. 2017;93:470–9.

82. Hu B, Xu C, Tian Y, Shi C, Zhang Y, Deng L, Zhou H, Gao P, Chen H, Yuan W. Inflammatory microRNA-194 and -515 attenuates the biosynthesis of chondroitin sulfate during human intervertebral disc degeneration. Oncotarget. 2017;8(30):49303–17.

83. Zhang B, Guo W, Sun C, Duan HQ, Yu BM, Mu K, Guan YY, Li Y, Liu S, Liu Y, et al. Dysregulated MiR-310a-3p promotes lumbar intervertebral disc degeneration by targeting aggrecan. Cell Physiol Biochem. 2018;45(6):2506–18.

84. Dong W, Liu J, Yu Y, Wang F, Liu T, Sun S, Liao B, Shu Z, Qian J. miR-640 aggravates intervertebral disc degeneration via NF-kappab and WNT signalling pathway. Cell Prolif. 2019;52(5):e12664.

85. Zhang Q, Weng Y, Jiang Y, Zhao S, Zhou D, Xu N. Overexpression of miR-143-5p inhibits lipopolysaccharide-induced human intervertebral disc inflammation and degeneration by downregulating Toll-like receptor 4. Oncol Rep. 2018;40(2):793–802.

86. Murillo OD, Thistlethwaite W, Rozowsky J, Subramanian SL, Lucero R, Shah P, Kohtz JD. The Evf-2 noncoding RNA targets miR-431 modulating IL-1beta-induced degradative changes in relevant tissues. J Mol Med (Berl). 2017;95(11):1179–89.

87. Wang X, Wang B, Zhou M, Li J, Lu G, Zhang Q, Liu F, Lu C. CircRNA-M84 targets miR-431 modulating IL-1β-induced degradative changes in nucleus pulposus cells in intervertebral disc degeneration via Wnt pathway. Biochim Biophys Acta Mol Basis Dis. 2018;1864(11):3754–68.

88. Li Z, Li X, Chen C, Li S, Shen J, Tse G, Chan MTV. Wu WK. Long non-coding RNAs in nucleus pulposus cell function and intervertebral disc degeneration. Cell Physiol Biochem. 2018;51(5):12483.

89. Feng J, Bi C, Clark BS, Mady R, Shah P, Kohtz JD. The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultrasensitive region and functions as a Dlx-2 transcriptional coactivator. Genes Dev. 2006;20(11):1470–84.

90. Yao Y, Li J, Wang L. Large intervening non-coding RNA HOTAIR is an indicator of poor prognosis and a therapeutic target in human cancers. Int J Mol Sci. 2014;15(10):18985–99.

91. Li X, Lou Z, Liu J, Li H, Lei Y, Zhao X, Zhang F. Upregulation of the long non-coding RNA IncPods contributes to intervertebral disc degeneration by negatively regulating RNA polymerase epsilon. Am J Transl Res. 2019;11(5):2843–54.

92. Han Z, Wang J, Gao L, Wang Q, Wu J. Aberrantly expressed messenger RNAs and long noncoding RNAs in degenerative nucleus pulposus cells co-cultured with adipose-derived mesenchymal stem cells. Arthritis Rheum Ther. 2018;20(1):182.

93. Zhao B, Liu M, Wang D, Li H, He X. Genome-wide identification of long noncoding RNAs in human intervertebral disc degeneration by RNA sequencing. Biomed Res Int. 2016;2016:3584875.

94. Wang Y, Song Q, Huang X, Chen Z, Zhang F, Wang K, Huang G, Shen H. Long noncoding RNA GASS promotes apoptosis in primary nucleus pulposus cells derived from the human intervertebral disc via Bcl2 downregulation and caspase3 upregulation. Mol Med Rep. 2019;19(2):1164–72.

95. Zhang Y, Wang X, Xiang Q, Song Y, Li S, Liang H, Luo R, Wang B, Liao Z, Zhang Y, et al. IncRNA HOTAIR upregulates autophagy to promote apoptosis and senescence of nucleus pulposus cells. J Cell Physiol. 2020;235(3):2195–208.

96. Shao T, Hu Y, Tang W, Shen H, Yu Z, Gu J. The long noncoding RNA HOTAIR serves as a microRNA-34a-5p sponge to reduce nucleus pulposus cell apoptosis via a NOTCH1-mediated mechanism. Gene. 2017;515:144029.

97. Tan H, Zhao L, Song R, Liu Y, Wang L. The long noncoding RNA SNHG1 promotes nucleus pulposus cell proliferation through regulating miR-326 and CCND1. Am J Physiol Cell Physiol. 2018;315(1):C27–7.

98. Wang X, Zou M, Li J, Wang B, Zhang Q, Liu F, Lu G. LncRNA H19 targets miR-22 to modulate H2 O2 -induced deregulation in nucleus pulposus cell senescence, proliferation, and ECM synthesis through Wnt signaling. J Cell Biochem. 2018;119(6):4990–5002.

99. Xu Y, Jiang T, Wang W, Yu J, Wang Y, Wu X, He Y. Long non-coding KCNH1 promotes intervertebral disc degeneration by sponging miR-146a-5p and regulating TRAF6 expression. Sci Rep. 2017;7(1):13234.

100. Zhao K, Zhai Y, Yuan H, Zhao M, Zhao D. Long noncoding RNA LINC00958 accelerates the proliferation and matrix degradation of the nucleus pulposus by regulating miR-203/SMA3D. Aging (Albany NY). 2019;11(23):10814–23.

101. Yang Y, Zhong Z, Zhou Y, Ren K, Li N. LincRNA-SLC20A1 (SLC20A1) contributes to extracellular matrix degradation in degenerative nucleus pulposus cell growth via targeting the Notch signaling pathway. J Cell Physiol. 2019;234(12):22163–71.

102. Zhao K, Maass PG, Glazar P, Memczak S, Dittmar G, Hollfinger I, Schreyer L, Sauer AV, Guo et al. Arthritis Research & Therapy (2020) 22:270
115. Deng RY, Hong T, Li CY, Shi CL, Liu C, Jiang FY, Li J, Fan XM, Feng SB, Wang YF. Long non-coding RNA zinc finger antisense 1 expression associates with increased disease risk, elevated disease severity and higher inflammatory cytokines levels in patients with lumbar disc degeneration. Medicine (Baltimore). 2019;98(52):e18465.

116. Mi D, Cai C, Zhou B, Liu X, Ma P, Shen S, Lu W, Huang W. Long noncoding RNA FAF1 promotes intervertebral disc degeneration by targeting the Erk signaling pathway. Mol Med Rep. 2018;17(2):3158–63.

117. Feng G, Zha Z, Huang Y, Li J, Wang Y, Ke W, Chen H, Liu L, Song Y, Ge Z. Sustained and bioreponsive two-stage delivery of therapeutic miRNA via Polyplex micelle-loaded injectable hydrogels for inhibition of intervertebral disc fibrosis. Adv Healthc Mater. 2018;7(21):e1800633.

118. Ma CJ, Liu X, Che L, Liu ZH, Samartzis D, Wang HQ. Stem cell therapies for intervertebral disc degeneration: immune privilege reinforcement by Fas/FasL regulating machinery. Curr Stem Cell Res Ther. 2015;10(4):285–95.

119. Wang HQ. Bring stem cell therapies to cure intervertebral disc degeneration to the forefront. Curr Stem Cell Res Ther. 2015;10(4):284.

120. Clarke LE, Richardson SM, Hoyland JA. Harnessing the potential of mesenchymal stem cells for IVD regeneration. Curr Stem Cell Res Ther. 2015;10(4):296–306.

121. Krock E, Rosenzweig DH, Haglund L. The inflammatory milieu of the degenerate disc: is mesenchymal stem cell-based therapy for intervertebral disc repair a feasible approach? Curr Stem Cell Res Ther. 2015;10(4):317–28.

122. Gantenbein B, Illien-Junger S, Chan SC, Walser J, Haglund L, Ferguson SJ, Iatridis JC, Grad S. Organ culture bioreactors--platforms to study human intervertebral disc degeneration and regenerative therapy. Curr Stem Cell Res Ther. 2015;10(4):339–52.

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