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Review Article

Preliminary construction of a regulatory network of miRNAs in the pathogenesis of nucleus pulposus degeneration - a review based on data mining

Yingjun Guo*, Xiaofei Wang*, Hao Liu, Beiyu Wang, Yang Meng, Chen Ding

Department of Orthopedic Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China.
**Equal contributors and co-first authors.*

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Abstract: In this study, we attempted to further collate existing transcriptome sequencing (mRNA-Seq) data by applying data mining and screening intervertebral disc degeneration (IVDD)-related miRNAs. At the same time, combined with published articles, the miRNAs that have been screened out were further excluded, and only the miRNAs confirmed by the reported studies were retained and reviewed. We obtained 12 pro-IVDD miRNAs and ten anti-IVDD miRNAs using the above screening process, involving 33 literature sources. By reviewing and summarizing the above studies, we preliminarily constructed the regulatory network of miRNA in the pathogenesis of IVDD. This regulatory network comprises many gaps and potential miRNA interactions, and these points may be the breakthrough points for further IVDD-related research. This new review approach can also provide a reference for the mechanistic studies of other diseases.

Keywords: Intervertebral disc degeneration, microRNA, nucleus pulposus, data mining

Introduction

Intervertebral disc degeneration (IVDD) is a common condition [1-3]. Although IVDD does not cause clinical symptoms in some cases, it remains detrimental to overall human health. The structure of the intervertebral disc (IVD) is relatively simple. The internal nucleus pulposus (NP) is surrounded by the annulus fibrosus (AF) and endplate (EP). NP tissue is the most important part that maintains the normal function of the IVD, in which NP cells (NPCs) play a critical role. However, the structure of the IVD is unique: it lacks a blood supply and innervation. Unlike other well-supplied organs and tissues, IVDD usually results from internal changes because IVD tissues lack a blood supply and are nourished mainly via EP osmosis. In addition, because of the lack of innervation in IVD tissues, no obvious symptoms are detected in the early stage of IVDD. Only when the IVD is enlarged or protruded because of degeneration, resulting in nerve tissue compression, will it be perceived by the patient. Therefore, the clinical management of IVDD is always delayed and passive.

Most of the preventive treatment of a disease should be based on the full understanding of its pathogenesis. Studies on the pathogenesis of IVDD have been ongoing, and several causes have been hypothesized, such as inflammation [4, 5], an oxidative stress response [4], altered osmotic pressure [6], inadequate nutrient supply [7], and an altered stress environment [8], each of which seemed to have a decisive influence. An increasing number of studies have reported that the construction of a regulatory network might better elucidate the mechanism of IVDD, and our study adds more elements and possibilities to the construction of this network. This concept is fully reflected in the relevant studies of IVDD and microRNAs (miRNAs).

The concept of non-coding RNAs (ncRNAs) and continuous improvement of the ncRNA regulatory system have provided new breaking points for many unsolved disease studies [1]. Among them, relevant miRNA studies have shed new

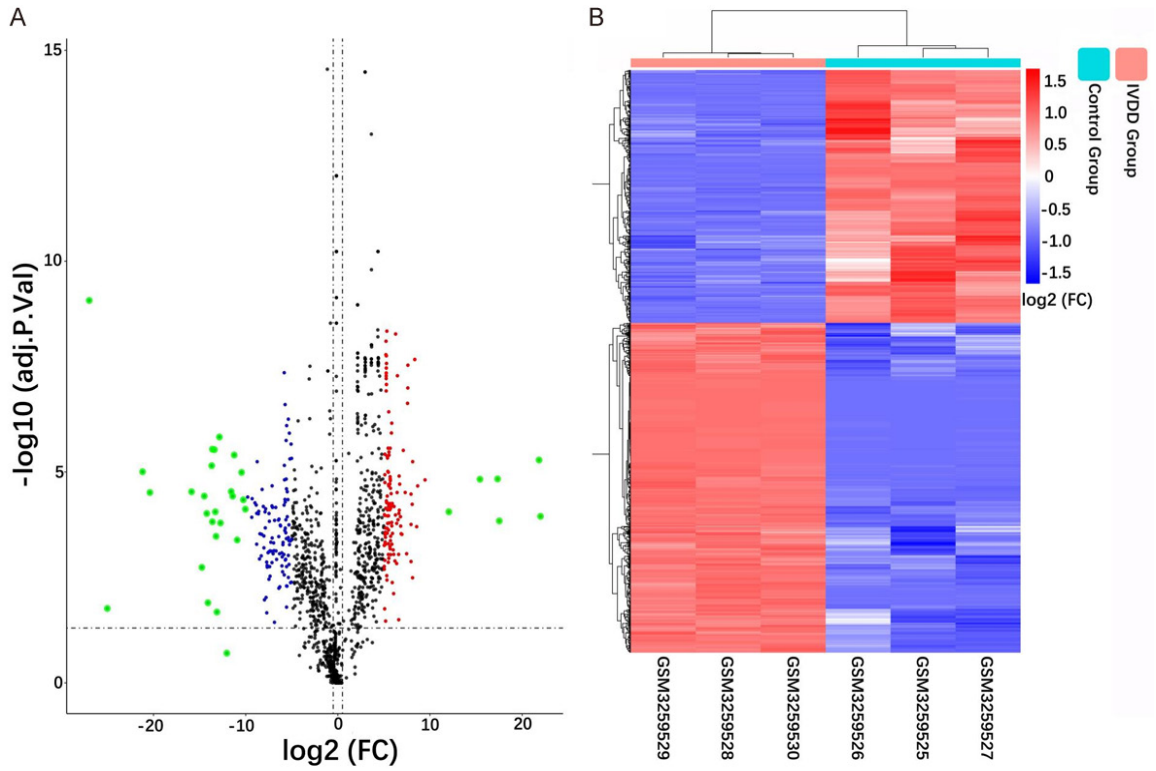

Figure 1. Different expression profiles of miRNAs in degenerated and non-degenerated human NP tissues. A. Volcano plot exhibit significantly dysregulated miRNAs in NP tissues. The horizontal lines represent 2-fold up or down (log2 FC>1 or log2 FC<-1) expressed miRNAs with the vertical lines indicate the *P* value is 0.05. Red and blue plots mark greatly dysregulated miRNAs (log2 FC>5 or log2 FC<-5). Green plots mark the miRNAs whose log2 FC>10 or <-10. B. Heat map exhibits the comparison of differentially expressed miRNAs in NP tissues between control group and IVDD group. Red and blue squares represent the up-regulation and down-regulation of miRNAs (P<0.05), respectively.

insights on the pathogenesis of many diseases, filling in many gaps between the extracellular environment and intracellular signaling pathway. In IVDD-related studies, miRNA regulation of NPC function has also become a new hot spot, and more than 100 related studies have been published to date. From these studies, a miRNA regulatory network for NPC function has emerged [3].

Recently, with the development of transcriptome sequencing (mRNA-Seq) technology, an increasing number of studies have screened miRNAs that may play a role in NPC degeneration, and many miRNAs with differential expression have been identified. In this study, the published mRNA-Seq results were first integrated using bioinformatics analysis, and the relevant contents of some miRNAs that have been verified in the literature were reviewed to confirm their authenticity. Thus, a signal network of miRNA regulation in NPC function could be initially constructed.

Data mining of published research

We searched the GEO database for the dataset of miRNA and IVDD (No. GSE116726). Using the "limma" extension package of R software, we screened a series of miRNAs with the following screening standards: "log Fold Change (FC) >1 or <-1" and "adjusted *P* value <0.05" (**Figure 1**). Generally, miRNAs with increased expression in IVDD tissues always represent pro-IVDD factors, and miRNAs with decreased expression will show protective roles in IVDD. However, this conclusion does not apply to all cases. For chronic degenerative diseases such as IVDD, feedback regulation is also possible. Therefore, we must further confirm the screened results according to the existing literature reports. Accordingly, we divided the miR-

Table 1. Bioinformatics screen of pro-IVDD miRNA and corresponding matching studies

miRNA	logFC	adj. P. Val	Related Physiological/Pathological Process	Reference
miR-21	21.8334557	5.13E-06	Proliferation	[10-12]
			Autophagy	
			ECM Anabolism & Catabolism	
			Inflammation	
miR-34a	17.5220332	0.000131278	ECM Anabolism & Catabolism	[14]
miR-146a-5p	15.4204769	1.44E-05	Apoptosis	[16]
			Proliferation	
			ECM Anabolism & Catabolism	
			Inflammation	
miR-125-1-3p	8.10468251	0.003160968	Proliferation	[19]
miR-15b	6.26646053	8.57E-09	ECM Anabolism & Catabolism	[21-23]
miR-27a	5.25246965	4.63E-08	Apoptosis	[27, 28]
			Inflammation	
miR-100	4.37704853	2.79E-08	ECM Anabolism & Catabolism	[34]
miR-132	4.33100976	1.34E-08	ECM Anabolism & Catabolism	[33]
miR-143-5p	4.37704853	2.79E-08	Apoptosis	[36, 37]
			Proliferation	
			Differentiation	
miR-30d	2.97256066	1.98E-08	Apoptosis	[40]
miR-154	2.9344262	1.23E-06	ECM Anabolism & Catabolism	[38]
miR-129-5p	2.14413035	3.01E-09	Apoptosis	[42]
			Autophagy	

Note: ECM, extracellular matrix.

NAs into pro-IVDD and anti-IVDD groups, as listed in **Tables 1** and **2**, respectively.

Matching review of mirna-related studies

Whether the NP tissue is normal directly affects the physiological function of the IVD, primarily depending on whether NPCs function normally. As chondrogenic cells, an important aspect of NPC function is the synthesis of proteins related to metabolism of extracellular matrix (ECM), such as type II collagen (Col II), aggrecan, matrix metalloproteinase (MMP), and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). In addition, normal cell processes such as proliferation, apoptosis, and autophagy greatly affect the function of the NP tissue. Therefore, the ultimate focus of most miRNA-related studies includes the aspects described above. According to the following review, the miRNA regulatory networks are shown in **Figures 2**-**4**, focusing on apoptosis/proliferation, ECM anabolism/catabolism, and inflammation, respectively.

Pro-IVDD miRNAs

miR-21: miR-21 is a very common miRNA expressed in several cancers, such as breast, lung, liver, and stomach cancers, and is involved in multiple cellular processes [9]. Chen et al. [10] reported that, by targeting programmed cell death 4 (PDCD4), miR-21 up-regulated the expression of MMP-2 and MMP-9 in NPCs through the c-Jun or activator protein-1 (AP-1) pathway. According to KEGG analysis, mitogen-activated protein kinase (MAPK) functions as upstream of PDCD4, but no direct correlation exists between miR-21 and MAPK. Wang et al. [11] also confirmed that miR-21 accelerated IVDD progression by promoting ECM degradation. They also found that miR-21, by silencing of phosphatase and tensin homolog deleted on chromosome ten (PTEN), activated the Akt/mTOR pathway to inhibit autophagy, leading to increased expression of MMP-3 and MMP-9, and subsequent degradation of Col II and aggrecan in human degenerated NP cells [11]. Lin et al. [12] reported that miR-21 inhibited

Table 2. Bioinformatics screen of anti-IVDD miRNA and corresponding matching studies

miRNA	logFC	adj. P. Val	Related Physiological/Pathological Process	Reference
miR-22	-13.393422	2.85E-06	Proliferation	[43]
			Cell Senescence	
			ECM Anabolism & Catabolism	
miR-486-5p	-11.362636	3.80E-06	Apoptosis	[45]
			Cell Viability	
			ECM Anabolism & Catabolism	
			Inflammation	
miR-10b	-8.2141161	2.59E-05	Proliferation	[49]
miR-140-5p	-7.6651036	0.001616618	ECM Anabolism & Catabolism	[51, 53]
			Inflammation	
miR-155	-7.0661984	7.46E-05	Apoptosis	[56, 58-60]
			ECM Anabolism & Catabolism	
miR-148a	-6.9191658	0.000717537	Inflammation	[61]
miR-223	-5.5615332	0.004943924	Apoptosis	[64]
			Proliferation	
			ECM Anabolism & Catabolism	
			Inflammation	
miR-93	-3.2783834	0.000652601	ECM Anabolism & Catabolism	[71]
miR-133a	-2.449575	0.000113326	ECM Anabolism & Catabolism	[72]
miR-194-5p	-1.4752873	0.001191302	Inflammation	[74]

Note: ECM, extracellular matrix.

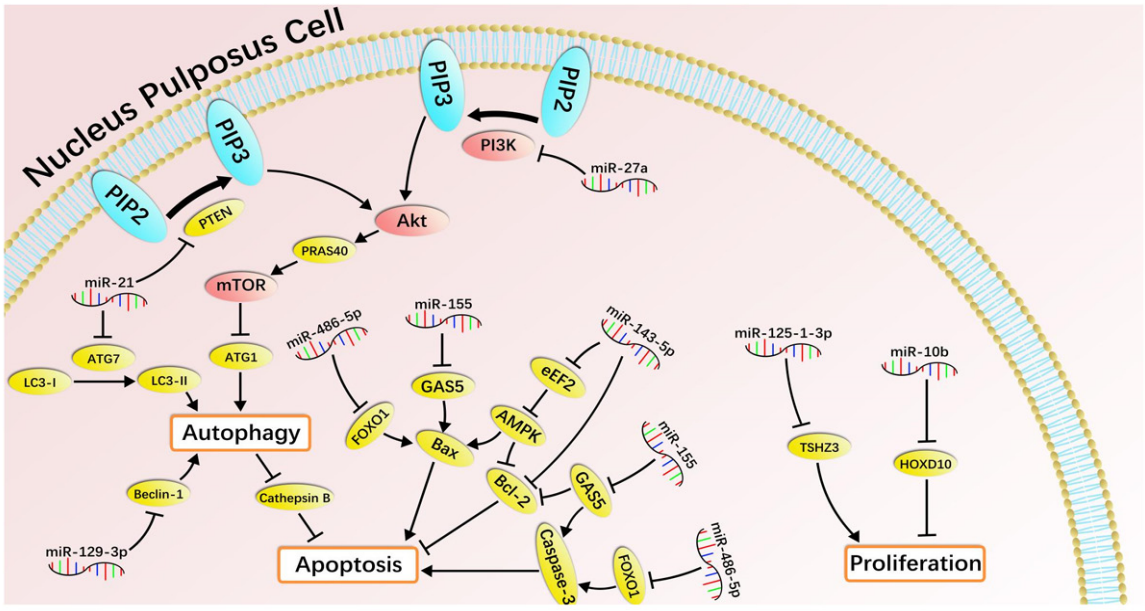

Figure 2. Apoptosis/Proliferation regulatory network in NPCs with miRNAs. miR-27a and miR-21 affect the activity of Akt pathway through targeted regulation of PTEN and PI3K respectively, and finally affect the autophagy through mTOR pathway. Meanwhile, miR-21 can also inhibit the autophagy by inhibiting the transformation of LC3-I to LC3-II. Similarly, miR-129-3p can also inhibit the occurrence of autophagy by inhibiting the expression of Beclin-1. All of the above miRNAs can indirectly regulate the process of apoptosis by affecting autophagy. On the other hand, miR-143-5p, miR-155 and miR-486-5p can indirectly regulate the expression of key apoptotic molecules such as Bax, Bcl-2 and Caspase-3 through different pathways, thus affecting the degree of apoptosis. miR-125-1-3p inhibits cell proliferation by inhibiting the expression of TSHZ3, whereas miR-10b promotes cell proliferation by inhibiting the expression of HOXD10.

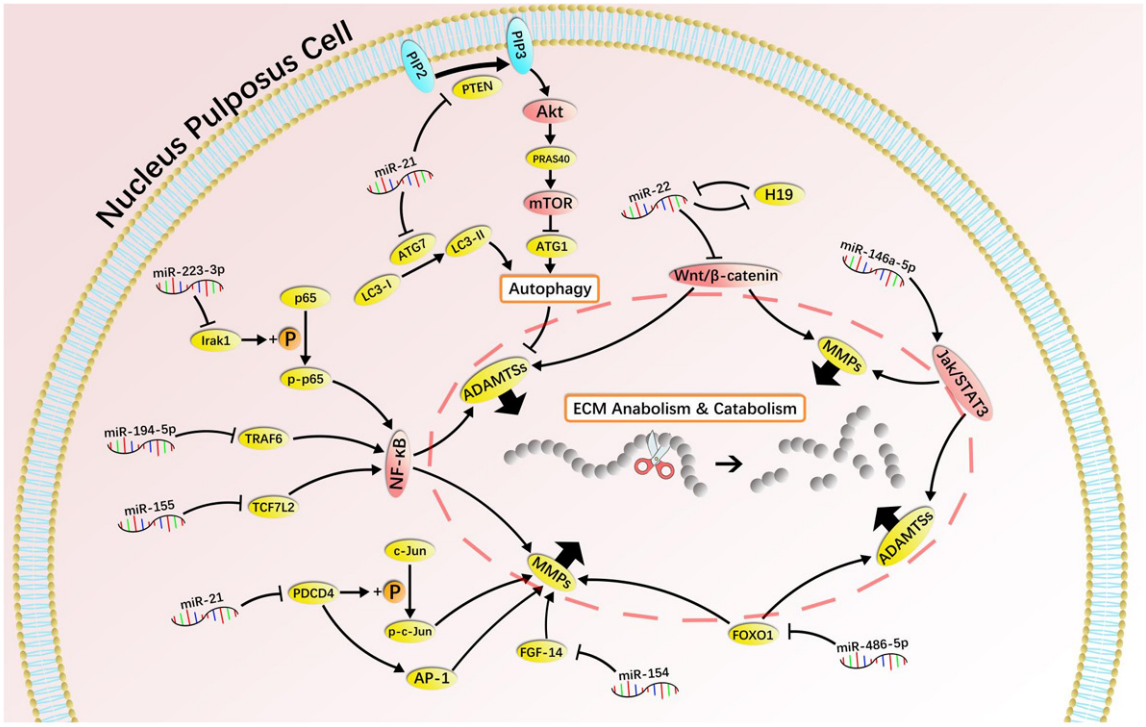

Figure 3. ECM Anabolism/Catabolism regulatory network in NPCs with miRNAs. miR-21, by targeting PTEN and inhibiting the transformation of LC3-I to LC3-II, inhibits autophagy and finally regulates ADAMTSs expression. Also, miR-21 can inhibit the expression of PDCD4, and then inhibit the phosphorylation of c-Jun and the activation of AP-1, finally affecting the expression of MMPs. miR-223-3p, miR-194-5p and miR-155 inhibit the activation of NF-κB through different pathways, and then regulate the expression of MMPs and ADAMTSS, respectively. miR-154 inhibits the expression of MMPs by targeting FGF-14. miR-486-5p inhibits the expression of MMPs and ADAMTSS via down-regulating the FOXO1 expression. miR-22 inhibits the activation of the canonical Wnt pathway through unknown mechanism, and ultimately inhibits the expression of MMPs and ADAMTSS. At the same time, there is mutual negative regulation between miR-22 and H19. miR-146a-5p activates the Jak/STAT3 also through the unknown pathway, thereby promoting the expression of MMPs and ADAMTSs. Finally, MMPs and ADAMTSs promote the degradation of key ECM molecules such as Col II and Aggrecan.

Col II and aggrecan synthesis and down-regulated the level of autophagy, possibly via its pro-inflammatory effect.

miR-34a: Current studies have confirmed that miR-34a plays an important role in regulating cartilage degeneration [13], based on which Liu et al. [14] provided evidence for the first time that miR-34a expression was up-regulated in NP tissues from patients with IVDD. Furthermore, by targeting growth differentiation factor 5 (GDF5), miR-34a silencing could induce Col II and aggrecan up-regulation in NP cells, a likely mechanism for its pro-IVDD effect [14].

miR-146a-5p: miR-146a-5p is one of the earliest miRNAs identified in cartilage and has been implicated in some degenerative diseases, including osteoarthritis [15]. Zhou et al. [16] reported that, by activating signal transducer and activator of transcription (STAT)-3 pathway, miR-146a-5p promoted the expression of interleukin (IL)-6, MMP-3, and ADAMTSs and inhibited Col II expression. However, they only provided the results of up-regulated STAT3 expression; no direct correlation was found between STAT3 and the differential expression of the above functional genes.

miR-125-1-3p: The functions of miR-125-1-3p have already been confirmed in tumor-related studies [17, 18]. Meng et al. [19] demonstrated that Teashirt zinc finger homeobox 3 (TSHZ3), which is a target gene of miR-125b-1-3p, might play a protective role in the degeneration and apoptosis of NP cells.

miR-15b: In previous studies, miR-15b was involved in acute liver failure, cardiac insuffi-

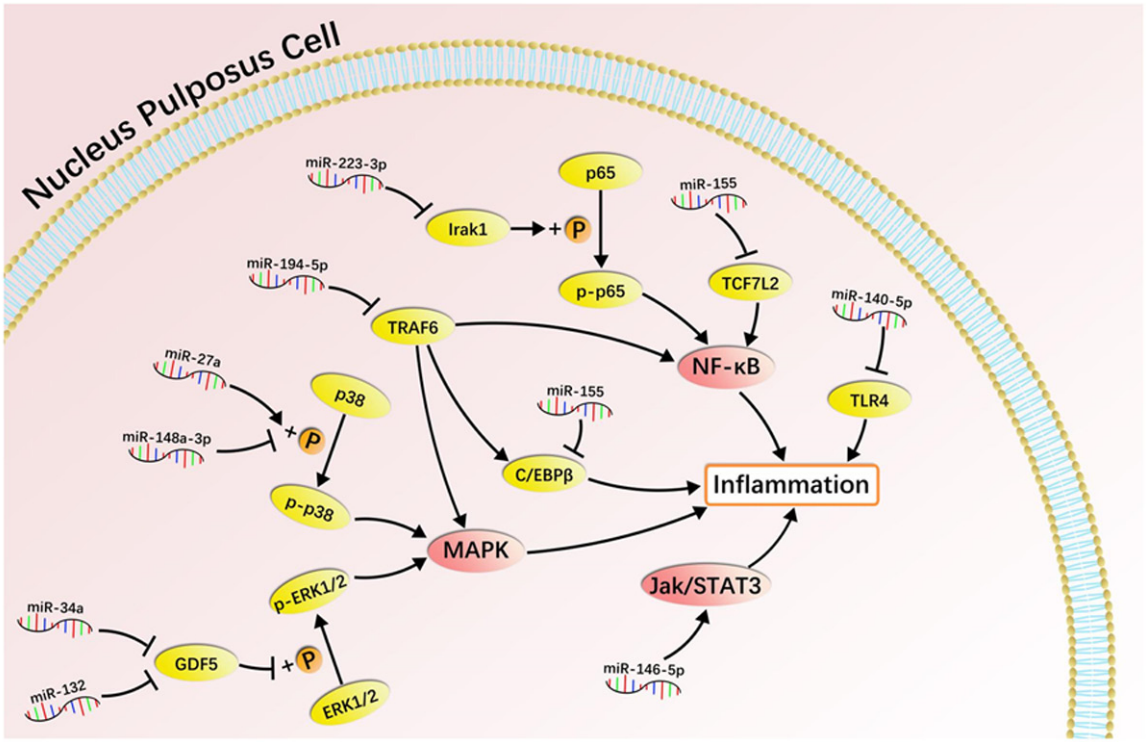

Figure 4. Inflammation regulatory network in NPCs with miRNAs. Both NF-κB and MAPK are classical inflammatory pathways, and most miRNAs in this regulatory network also indirectly regulate inflammatory response through these two pathways. miR-223-3p and miR-155 inhibit the activity of NF-κB by inhibiting p65 phosphorylation and TCF7L2 expression, respectively. miR-27a and miR-148a-3p can promote and inhibit p38 phosphorylation, respectively, and further regulate MAPK pathway activity. At the same time, both miR-34a and miR-132 can promote the phosphorylation of ERK1/2 by inhibiting GDF5, and finally promote the activity of MAPK pathway. miR-194-5p can inhibit the activity of the above two pathways by targeting TRAF6, while miR-155 can directly target inhibition of C/EBPβ, and then enhance the inhibition ability of miR-194-5p on TRAF6-C/EBPβ-inflammation axis. miR-140-5p can inhibit the expression of TLR4, and then reduce the severity of inflammation. miR-146a-5p activates the Jak/STAT3 through the unknown pathway, thereby promoting inflammation.

ciency, and breast cancer by regulating cell proliferation, differentiation and apoptosis [20-22]. Kang et al. [23] reported that miR-15b up-regulation in the degeneration of NP tissues and its silencing inhibited IL-1β-induced ECM degradation by targeting mothers against decapentaplegic homolog (Smad)-3 mRNA. Furthermore, miR-15b up-regulation is mediated by the NF-κB and MAPK pathway.

miR-27a: As a multifunctional miRNA, miR-27a is expressed in various tissues and cells, and the abnormal expression of miR-27a is closely related to the occurrence and development of various diseases [24-26]. Liu et al. [27] reported that miR-27a was up-regulated in damaged NP cells, and, by directly targeting the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, miR-27a promoted apoptosis in NPCs from IVDD. Furthermore, Cao et al. [28] presented that miR-27a down-regulation suppressed the inflammatory response in NPCs via the p38/MAPK pathway. Both studies above highlight the possibility that miR-27a is a novel potential therapeutic target for IVDD.

miR-132: As one of the most studied miRNAs, miR-132 is associated with multiple cellular processes [29-31], and abnormal miR-132 expression is involved in the pathological mechanism of osteoarthritis [32], while the expression and function of miR-132 in IVDD remain unknown. Liu et al. [33] reported that miR-132 promoted the expression of MMP-13 and ADAMTS-4 and inhibited the expression of Col II and aggrecan in IVDD by directly targeting GDF5, an activity that is mediated by the MAPK pathway.

miR-100: Yan et al. [34] reported that miR-100 up-regulation in lumbar disc degeneration was inversely correlated with the expression level of

fibroblast growth factor receptor 3 (FGFR3). Additionally, miR-100 targeted FGFR3 mRNA to down-regulate its expression, leading to MMP-13 activation and IVDD.

miR-143-5p: Few relevant studies have investigated miR-143-5p, which plays an important role in epithelial-mesenchymal transformation and metastasis of gallbladder carcinoma [35]. Yang et al. revealed that miR-143-5p expression was up-regulated in degenerative NP tissues [36], activating the adenosine monophosphate activated protein kinase (AMPK) pathway by targeting eukaryotic elongation factor 2 (eEF2), to induce NPC apoptosis and senescence. However, Zhao et al. [37] demonstrated that miR-143-5p overexpression induced a decrease in the number of NP cells by reducing B-cell lymphoma-2 (Bcl-2) expression in human degenerative NPCs, accelerating NPC apoptosis. These results suggest that miR-143-5p may play a pivotal role in the pathogenesis of IVDD and may become a candidate to develop miRNA-based treatments.

miR-154: Previous studies have shown that miR-154 is involved in numerous cell processes [38, 39]. Wang et al. [35] found that miR-154 expression was up-regulated in the NPCs of IVDD by targeting fibroblast growth factor 14 (FGF14), and miR-154 up-regulated the expression of MMP-13 and ADAMTS-4 and downregulated the expression of Col II and aggrecan.

miR-30d: miR-30d is involved in apoptosis regulation and bone homeostasis, and Lv et al. [40] reported that miR-30d was up-regulated in human degenerative NP tissues. By targeting SRY-box transcription factor 9 (SOX9), miR-30d inhibits the viability and promotes the apoptosis of NPCs; furthermore, miR-30d up-regulates the expression of MMP-3 and MMP-13 and downregulates the expression of Col II and aggrecan [40]. Therefore, approaches to modulate miR-30d/SOX9 have potential applications in IVDD treatment.

miR-129-5p: The methylation of miR-129-5p in osteosarcoma is associated with the expression of human valine-containing proteins [41]. Based on these findings, Zhao et al. [42] speculated that miR-129-5p regulates IVDD progression via DNA methylation. Furthermore, by targeting Beclin-1, miR-129-5p inhibits autophagy in degenerative NPCs. Autophagy, according to Zhao's work [42], protects NPCs from apoptosis by inhibiting cathepsin B; thus, miR-129-5p accelerates IVDD through the beclin-1/autophagy/cathepsin B pathway.

Anti-IVDD miRNA

miR-22: Wang et al. [43] reported that miR-22, which undergoes negative regulation by H19, exerted a protective effect in NPCs by down-regulating the protein levels of lymphoid enhancing factor-1 (LEF1), Myc, and Cyclin D1. Additionally, they reported that miR-22 also inactivates the Wnt signaling pathway, ultimately inhibiting the expression of MMPs and ADAMTSs, and protecting the ECM of NP tissues from degradation.

miR-486-5p: Notably, Ji et al. found that the level of miR-486-5p was significantly lower in IVDD samples compared with that in controls [44]. In the recent study of Chai et al. [45], they found that, under lipopolysaccharide (LPS) induction, miR-486-5p, by targeting the forkhead box protein O1 (FOXO1), promoted the expression of Col II, aggrecan, and Bcl-2 but inhibited the expression of MMP-3, MMP-13, ADAMTS-4, ADAMTS-5, Bax, and Caspase-3.

miR-10b: miR-10b is one of the most well-studied miRNAs involved in regulating cell proliferation [46, 47], and it is expressed in diverse tissue types [48]. Yu et al. [49] found that up-regulated miR-10b expression correlated with the degeneration grade in IVDD. Furthermore, miR-10b induced NPC proliferation by activating Rho-Akt signaling through targeting homeobox protein D10 (HOXD10).

miR-140-5p: miR-140-5p is a cartilage-specific miRNA that plays a major role in pathogenesis [50, 51]. In a recent study, miR-140-5p was found to protect chondrocytes against the anti-proliferation and cell-matrix signaling changes induced by IL-1β [52]. Yang et al. [53] recently reported that IL-1β-or tumor necrosis factor (TNF)-α induced ECM degradation by inhibiting miR-140-5p; normally, miR-140-5p promotes Col II expression by targeting cyclic adenosine monophosphate (cAMP) responsive element binding protein 1 (CREB1). Zhang et al. [54] found that miR-140-5p overexpression inhibit-

ed the inflammatory response by down-regulating toll-like receptor TLR4.

miR-155: Previous reports have revealed that abnormal miR-155 expression plays a critical role in regulating ECM degradation in degenerative diseases, including IVDD and osteoarthritis [55-58]. Sun et al. [59] found that transcription factor 7-like 2 (TCF7L2) promoted ECM degradation via the NF-κB signaling pathway, which could be repressed by miR-155. Ye et al. [58] revealed that miR-155 inhibition decreased the expression of extracellular matrix Col II and aggrecan by promoting the expression of extracellular signal-regulated kinase 1/2 (ERK1/2). Furthermore, Zhou et al. [56] reported that miR-155 acted as a sustainable factor in IVD to suppress the expression of catabolic genes induced by inflammation by targeting CCAAT/enhancer binding protein β (C/EBPβ) in NPCs. Wang et al. [60] found that miR-155 downregulated Caspase-3 expression and up-regulated Bcl-2 expression by targeting growth arrest-specific transcript 5 (GAS5).

miR-148a: The recent study by Li et al. [61] suggests that miR-148a functioned in IVDD progression, and miR-148a overexpression suppressed the release of proinflammatory factors, including TNF-α, IL-1β, and IL-6, by inactivating the p38/MAPK pathway.

miR-223: Previous results have shown that miR-223 is involved in mediating macrophage inflammatory responses [62]. Another study showed that miR-223 downregulates interleukin-1 receptor-associated kinase 1 (Irak1) to mediate inflammatory responses in macrophages [63]. Wang et al. [64] found that, by suppressing NF-κB signaling through targeting Irak1, miR-223 up-regulated the expression of Col II and aggrecan and downregulated the expression of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5. Furthermore, miR-223 also inhibited the up-regulation of LPS-induced nitric oxide (NO) reaction-associated genes, including cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), and prostaglandin E2 (PGE2).

miR-93: Previous studies have shown that miR-93 plays a role in multiple cellular processes [65-68], whose expression is down-regulated in various tumors, such as hepatocellular carcinoma, colon cancer, non-small lung cancer and gastric cancer [68-70]. Jing et al. [71] reported that miR-93 expression in human degenerative NP tissues was negatively correlated with the degree of IVDD, while miR-93 overexpression up-regulated Col II expression by targeting MMP-3.

miR-133a: Using mRNA-Seq, Xu et al. [72] reported that miR-133a is markedly downregulated in IVDD tissues. They also demonstrated that miR-133a, by targeting MMPs, protects Col II from degradation.

miR-194-5p: The expression of miR-194-5p in cord blood granulocytes of newborn infants is down-regulated after LPS induction, suggesting a role for miR-194-5p in the LPS-stimulated TLR signaling pathway [73]. Another study reported that miR-194-5p regulated the palmitic acid-induced TLR4 inflammatory response by targeting TNF receptor-associated factor 6 (TRAF6) in human THP-1 cells [73]. Furthermore, miR-194-5p regulated the LPS-induced NF-κB signal by mediating TLR4 expression. Collectively, miR-194 is a critical miRNA involved in LPS-induced inflammatory responses. Chen et al. [74] reported that cullin (CUL) 4A and CUL4B were specifically over-expressed in IVDD tissue, and miR-194-5p repressed both CUL4A and CUL4B expression. Kong et al. [75] found that miR-194-5p was decreased in the LPS-induced NPC, and miR-194-5p overexpression inhibited NF-κB signaling pathway activation by targeting TRAF6, leading to the suppression of LPS-induced inflammatory responses and ECM degradation.

Construction of regulatory networks

As mentioned above, we constructed three regulatory networks with the existing results according to the different cellular functions. In fact, most of the relevant studies have focused on the viability (**Figure 2**) and synthesis (**Figure 3**) of NPCs. What is shown in **Figure 4** is the regulation of miRNAs for the inflammation, which is more like the upstream “master switch” of cell function for NPCs. Cell functions including apoptosis, proliferation, senescence, differentiation, and ECM anabolism/catabolism are all regulated by degree of inflammation. However, this is not the key point of our discussion in this review, and we should emphasize more on how miRNAs are involved in the above cellular processes.

Conclusion

The construction of the regulatory network of miRNAs in the pathogenesis of IVDD mainly includes three parts: (1) screening of related miRNA; (2) verification of regulatory functions of related miRNA; and (3) series and parallel analyses of each miRNA regulatory pathway. Because of its unique regulation mode, miRNAs can directly participate in all aspects of the IVDD regulation network [3]. The higher the position of miRNA's action link, the more diversified is its action direction, but the regulatory effect of each direction may be weak. By contrast, the lower the position of the action link of miRNA, the more monotonous is its action direction, but its regulatory effect on the function of NPCs may be relatively strong. Therefore, after establishing the regulatory network of miRNA in IVDD, we can observe the hierarchical relationship of different miRNAs more clearly and identify existing gaps in the regulatory network, which is the potential breakthrough for further study. We can also search for possible linkages between miRNAs, i.e., mutual regulation-which may explain why certain signaling pathways have two-sided regulatory effects on IVDD, such as Wnt/β-catenin. Unfortunately, clinical translational studies on miRNA and IVDD are relatively rare, and only 2 of the 33 articles included in this review describe this aspect.

As a new approach to a review article, this study has many shortcomings. First, only one set of mRNA-Seq data was selected in the data mining, and the authenticity and comprehensiveness of the screening results were limited. Second, because of the small number of studies on AF and EP, this review only integrated the relevant studies on NPCs. Third, the current review did not consider other ncRNAs, such as lncRNAs and circRNAs, a finding that will continue to be supplemented and improved in subsequent work.

This review may provide a new strategy for related studies on other diseases by combining the results of data mining with those of related studies. Additionally, this study emphasizes the importance of building a regulatory network. Taking IVDD in this paper as an example, we believe that the reconstruction of a relatively stable miRNA environment may achieve a better IVDD prevention effect than the regulation of a single miRNA.

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Disclosure of conflict of interest

None.

Abbreviations

ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AF, annulus fibrosus; AMPK, adenosine monophosphate activated protein kinase; AP-1, activator protein-1; Bcl-2, B-cell lymphoma-2; cAMP, cyclic adenosine monophosphate; Col II, type II collagen; COX, cyclooxygenase; CREB1, cAMP responsive element binding protein 1; CUL, cullin; C/EBPβ, CCAAT/enhancer binding protein β; DM, data mining; ECM, extracellular matrix; eEF2, eukaryotic elongation factor 2; EP, endplate; ERK1/2, extracellular signal-regulated kinase 1/2; FC, fold change; FGF14, fibroblast growth factor 14; FGFR3, fibroblast growth factor receptor 3; FOXO1, forkhead box protein O1; GAS5, growth arrest-specific transcript 5; GDF5, growth differentiation factor 5; HOXD10, homeobox protein D10; iNOS, inducible nitric oxide synthase; Irak1, interleukin-1 receptor associated kinase 1; IVD, intervertebral disc; IVDD, intervertebral disc degeneration; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MMP, matrix metalloproteinase; mRNA-Seq, transcriptome sequencing; ncRNA, non-coding RNA; NP, nucleus pulposus; NPC, nucleus pulposus cell; PDCD4, programmed cell death 4; PGE2, prostaglandin E2; PTEN, phosphatase and tensin homolog deleted on chromosome ten; SMAD, mothers against decapentaplegic homolog; SOX9, SRY-box transcription factor 9; TCF7L2, transcription factor 7-like 2; TLR, toll-like receptor; TNF,

tumor necrosis factor; TRAF6, TNF receptor-associated factor 6; TSHZ3, teashirt zinc finger homeobox 3.

Address correspondence to: Hao Liu, Department of Orthopedic Surgery, West China Hospital, Sichuan University, No. 37 Guoxue Road, Chengdu 610041, Sichuan, China. Tel: +86-18980601369; E-mail: hxliuhao@126.com

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