Research Article

miR-328-5p Induces Human Intervertebral Disc Degeneration by Targeting WWP2

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1. Introduction

With the increasing incidence of low back pain (LBP), it has become the most important trigger to disability worldwide, which has brought a tremendous economic pressure [1–3]. The cause of LBP is very complex. And some known factors affect the advancement of LBP include genes, age, and lousy living habits (such as occupation, smoking, trauma, and mechanical loading) [4, 5]. Furthermore, it is believed that the main cause of LBP is IDD [6–8]. As a bridge between adjacent vertebral bodies, intervertebral disc includes the nucleus pulposus (NP), annulus fibrosus, and the cartilaginous endplate [9]. The most important pathological feature of IDD is the apoptosis of nucleus pulposus cells [10–13]. The apoptosis of nucleus pulposus cells triggers the progress of IDD [14–16], and this affects clearly the disc structure balance. Some studies suggested that abnormal apoptosis is associated with degenerative diseases such as osteoarthritis, IDD, and cancer [17–19].

However, the current treatment for IDD is limited to symptomatic intervention and cannot completely improve the prognosis of the disease [20]. Many studies confirmed...
that some regulatory genes have an essential impact in the incidence and development of IDD, such as microRNA (miRNA). The miRNA are single-stranded noncoding small RNA with 18 to 24 bp nucleotide sequences, which participates in regulating the cell proliferation and apoptosis [21–25]. And miRNA can negatively regulate the posttranscriptional gene expression in different species by either inhibiting mRNA translation or promoting mRNA degradation [25, 26]. Previous studies have also found that miRNA affects the progress of IDD by facilitating inflammatory response, cell apoptosis, and degradation of extracellular matrix [27]. Furthermore, miRNA has contributed to cardiovascular disease, cancer, leukemia, and skeletal muscle diseases [28]. Therefore, further research on the mechanism of miRNA regulation of IDD may lead to new therapeutic directions.

Some studies have found miR-328-5p is atypically expressed in lung cancer, breast cancer, and other tumors. In addition, the researcher indicated miR-328-5p was related with the proliferation and apoptosis of cancer cells [29–32]. Furthermore, studies have shown dissimilarity expression of WWP2 in various diseases such as oral cancer, endometrial cancer, ovarian cancer, glioma, and lung cancer by regulating cell apoptosis [33–38]. Both miR-328-5p and its target gene WWP2 can affect cell proliferation and apoptosis, and the mechanism of miR-328-5p mediating WWP2 regulating IDD has not been reported. This series of studies aims to research the mechanism of the above regulatory pathways and whether they are involved in the formation of IDD by inducing nucleus pulposus cell apoptosis.

### 2. Materials and Methods

#### 2.1. Clinical Sample Collection

We obtained 20 human NP samples via surgical discectomy. Surgical indications: (1) failure of conservative treatment and (2) progressive neurological deficits. Patients were excluded with isthmus or degenerative spondylolisthesis, ankylosing spondylitis, and diffuse idiopathic hyperostosis. According to T2-weighted midsagittal pffirmann disc degeneration grading criteria [39]. Grade I–II are normal intervertebral discs, and grade III–V are degenerative intervertebral discs. Table 1 presents the clinical features of patient.

#### 2.2. Isolation and Culture of NP Cells

The NP tissue was separated from the intervertebral disc, made into 2-3 mm³ sections under the microscope in aseptic conditions, and
Figure 2: Assessment of different mRNAs expressions in normal and degenerative nucleus pulposus tissues. (a) a volcano plot of mRNAs expression by $|\log FC| > 1, |P.V \text{ value}| < 0.05$ as selection criteria. (b) heatmap for hierarchical clustering of selected mRNA expression. (c and d) GO, and KEGG functional annotations were performed on the differentially screened mRNAs, respectively. The bar chart represented GO enrichment analysis, while the bubble chart represented KEGG enrichment analysis.
washed three times with PBS. Then prepared tissues were digested with 0.25 mg/mL type II collagenase (Invitrogen; Thermo Fisher Scientific) at 37°C for 12 h in Dulbecco’s Modified Eagle Medium (GIBCO, NY, USA). Then NP cells isolated from NP tissues by trypsin were placed in DMEM/F12 incubated at 37°C with 5% CO₂. The second generation of cells was chosen for the following experiment.

2.3. Transfection of NP Cells. The miR-328-5p mimic/inhibitor, mimi/inhibitor control, WWP2 siRNA, and siRNA control were derived from Sangon (Shanghai, China). In our study, miR-328-5p mimic/inhibitor promote/inhibit the expression of miR-328-5p, and mimic/inhibitor control were used as control groups, respectively. WWP2 siRNA inhibits WWP2 expression in the NP cells. According to the reagent instructions, RNA transmate was used to transfect the NP cells (Sangon, Shanghai, China). The cells were collected 48 hours later for subsequent experiments.

2.4. qRT-PCR. General RNA was obtained from NP tissue or cultured NP cells using TRIZON (TianGen, Beijing, China) according to reagent instructions. We quantified miRNA and mRNA expression levels in NP tissues or cells by LightCycler 480 II (Roche Diagnostics, Indianapolis, USA). The PCR reaction system of 20 ul contained 10 ul Universal SYBR Green Fast qPCR Mix, 2 ul cDNA, 0.8ul primers, and 7.2 ul nuclease-free water. The reaction conditions: 95°C for 15 min, 40 cycles for 10 s at 95°C, and 60°C for 20s. The experiment was performed three times. Experiment is based on U6 and β-actin [40]. Gene expression was measured using the 2^-ΔΔCt. Primers are as follows: miR-328-5p forward: AACGAGACGACGACAGAC, reverse: GGGGGGGGCGGGGCTCAGGG; WWIP2 forward: GAGATGGACAACGAGAAG, reverse: CTCCTCAATGGCATACAG; Bcl-2 forward: CACGAGAGGTCTTTTTCCGAG, reverse: CCAGCCCATGATGGTTCGAG; Bax forward: ATGGTTTGAGCCGAGCTTCTGAG; Caspase3 forward: ATGGTTTGAGCCGAGCTTCTGAG; etc.

Figure 3: Interaction between differential miRNAs and their downstream target gene mRNAs: (a) the intersection of three datasets, (b) 27 different mRNAs, and (c) cytoscape analyzed the network diagram.
CTGACGACAGA, reverse: GGCAGCATCATCCACACATAC; U6 forward: CTCGCTTCGGCAGCACA, reverse: AACGCT TCACGAATTTGCCT; β-actin forward: AGGGGCCGGAC TCGTCACT, reverse: GGCGGCACCACCATGTACCCT.

2.5. Western Blot. The protein was obtained with RIPA and BCA (Beyotime, Shanghai, China) measures protein concentration. The NC membrane was blocked with 5% skimmed milk at room temperature for 2 h, then washed three times with TBST and added overnight to primary antibody at 4°C. The primary antibodies: anti-WWP2 antibody (Proteintech, Wuhan, China), anti-Bax antibody (Cell Signaling, Danvers, MA, USA), anti-Bcl-2 antibody (Cell Signaling, MA, USA), anti-Caspase3 antibody (Cell Signaling, MA, USA), and anti-beta-actin antibody (Proteintech, Wuhan, China). After primary antibody incubation, the NC film was washed three times with TBST and added to goat anti-rabbit or mouse antibody (Vicmed, Xuzhou, China) for 2 h at room temperature in the dark. Beta actin was selected as the internal control [40].

2.6. CCK8. The cultured NP cells were transferred on 96-well plates by $2 \times 10^5$ cells/well, and added with miR-328-5p

![Western Blot Diagram](attachment:western_blot_diagram.png)

**Figure 4**: Correlation and differentially expression of miR-328-5p and WWP2. (a) the network diagram between miR-328-5p and its downstream genes. (b, c, and d) the correlation between miR-328-5p and its target genes. (e and f) significant differences in miR-328-5p and WWP2 expression. *P < 0.05, **P < 0.01.
mimic/inhibitor, and miR-control. Subsequent to the incubation, the cell culture medium was changed at 0 h, 12 h, 24 h, and 36 h of each well. 10 ul CCK8 reagent and 90 ul DMEM were added into every well and incubated for another 2 h at 37°C by the CCK8 kit (Vicmed, Xuzhou, China). The optical density was measured at 450 nm, and the experiment was repeated three times for each group.

2.7. Flow Cytometry. The detection of NP apoptosis was carried out by the flow cytometry instructions (KeyGEN, Nanjing, China). First, the transfected NP cells were separated in 0.25% trypsin (without EDTA) (Vicmed, Xuzhou, China). After rinsing the cells twice with PBS, 1 × 10⁶ cells were collected by centrifugation for 5 min at 2000 rpm. It is essential to rinse off as much residual trypsin digestive fluid as possible. After suspension of 500 ul binding buffer, each
Figure 6: Continued.
centrifugation tube was added 5 ul AnnexinV-APC and 5 ul PI. The samples were thoroughly mixed, and the reaction was carried out at room temperature in the dark for 5-15 min. Finally, flow cytometry was used for observation and detection. Three experiments are required.

2.8. Luciferase Reporter Assay. In order to construct wild or mutant-type expression vectors, the WW2P3′-UTR binding to miR-328-5p was inserted into the GV272 vector. Then, NP cells were added with wild type (Wt) or mutant type (Mut) WW2P3′-UTR reporter plasmid and miR-328-5p mimic. Luciferase enzyme activity was detected according to Promega (Madison, WI, USA) reagent instructions after transfection 48 h. The luciferase enzyme activity was normalized to renilla luciferase activity. And western blot was used to detect WW2P protein expression. The experiment was performed three times.

2.9. Statistical Analysis. Statistical was analyzed by the SPSS 26 (SPSS, Chicago, USA). GraphPad Prism 8.4 (GraphPad Software, CA, USA) was used for graphical representation. Mean ± SD was used to analyze the experimental data. t-test or one-way ANOVA was used for inter-group data analysis. Pearson’s test was used for correlation analysis. P < 0.05 indicates statistical difference.

2.10. Ethics Statement. The Ethics Committee of Huai’an Affiliated Hospital of Xuzhou Medical University approved this study. Human NP tissue samples were obtained from patients who underwent surgery in Huai’an Affiliated Hospital of Xuzhou Medical University from September 2020 to April 2021. Meanwhile, the patients’ written consent was informed, and the tissue samples were obtained during the operation.

3. Results

3.1. Assessment of Differentially Expressed miRNAs. A volcanic map of the dataset (GSE63492) showed that some miRNAs are differentially expressed between normal and degenerated nucleus pulposus tissues among the 31 miRNAs, hsa-miR-328-5p, and hsa-miR-183-3p expression were upregulated. And 21 miRNAs expression, including miR–486-5p, hsa-miR-486-5p were downregulated (Figure 1(a)). Heatmap figure shows that individual miRNA expressions differed significantly (Figure 1(b)).

3.2. Assessment of Differentially Expressed mRNAs. The volcano map analysis database (GSE34095) obtained a pairwise comparison of mRNA expression between normal and degenerative nucleus pulpos tissues. The experiment identified 348 differentially expressed upregulated genes such as TGFBI and PDGFC, while 260 downregulated genes such as WW2P and MPST (Figure 2(a)). Further, the stratified clustering analysis of intervertebral disc dataset using heatmap revealed differences in the expression of some genes (Figure 2(b)). GO including molecular function, cellular component, and biological process, and KEGG enrichment analysis was performed for differentially expressed mRNA using R language (Figures 2(c) and 2(d)).

3.3. Interactions between miRNA and mRNA. In order to analyse the relationship between mRNAs and miRNA, we go through miRTarBase (https://www.mirbase.org), TargetScan (http://www.targetscan.org/), and miRDB (http://mirdb.org/index.html) database to predict the miRNA target gene. The intersection of threedatasets was obtained by the Venn diagram representing 35 miRNAs and their downstream 699 target gene mRNAs (Figure 3(a)). And 27 different mRNAs containing WW2P were obtained by predicting intersection mRNAs via Venn diagram (Figure 3(b)). Furthermore, cytoscape analyzed the network diagram of 27 differential mRNAs and their upstream miRNAs (Figure 3(c)).
Figure 7: Continued.
significantly (Figures 4(e) and 4(f)). The most important pathological feature of IDD is the apoptosis of nucleus pulposus cells [10–13]. Bioinformatics analysis shows that WWP2 is a downstream target gene of miR-328-5p. The conditions of target genes we selected were as follows: high connectivity of network diagram, negative correlation between miRNA and its downstream target mRNA, regulate cell apoptosis, and at the same time, relevant literature was reviewed to understand the function of miRNA and mRNA, so we selected miR-328-5p-WWP2 pathway as our research object. We hypothesize that miR-328-5p regulates IDD development by mediating WWP2.

3.5. Differentially Expressed of Relevant miRNA and mRNA in Degenerative and Normal Nucleus Pulposus Tissues. Experiment confirmed that miR-328-5p gene expression was upregulated ($P < 0.01$, Figure 5(a)). Spearman’s correlation found that miR-328-5p expression was positively correlated with IDD grade ($R = 0.889$, $P < 0.001$, Figure 5(b)). WWP2 expression was downregulated ($P < 0.05$, Figure 5(c)), and the Spearman’s correlation of WWP2 expression was negatively correlated with IDD grade ($R = -0.929$, $P < 0.001$, Figure 5(d)). Pearson’s correlation between miR-328-5p and WWP2 gene expression was significantly negative ($R = -0.92$, $P < 0.001$, Figure 5(e)). Bax and Caspase3 expression was significantly increased in the degenerative nucleus pulposus tissues, while Bcl-2 was decreased ($P < 0.05$, Figure 5(f)).

3.6. MiR-328-5p Regulates WWP2 Expression and Promotes Apoptosis of NP Cells. MiR-328-5p mimic induced miR-328-5p, Bax and Caspase3 gene expression obviously, while WWP2 and Bcl-2 were downregulated compared to the control group (Figures 6(a), 6(b), and 6(c)). miR-328-5p mimic significantly inhibited the protein expression of WWP2, Bcl-2 and promoted Bax and Caspase3 expression, while the miR-328-5p inhibitor obtained the opposite result (Figures 6(d), 6(e), and 6(f)). CCK8 assay confirmed that miR-328-5p mimic significantly inhibited the proliferation of NP cells (Figure 6(g)). Flow cytometry identified that the apoptosis of NP cells increased obviously after the addition of miR-328-5p mimic (Figure 6(h)). In conclusion, the above experimental results suggest that miR-328-5p can induce NP cells apoptosis.

3.7. MiR-328-5p Promotes the Apoptosis of NP Cells by Directly Targeting WWP2. The luciferase activities were decreased after the cotransfection of wild type (Wt) WWP2 $3′$-UTR reporter plasmid and miR-328-5p mimic into NP cells (Figure 7(a)). The corresponding sequence of WWP2 $3′$-UTR plasmids Wt or Mut and miR-328-5p was enumerated (Figure 7(b)). WWP2 protein expression was downregulated after cotransfecting Wt and miR-328-5p mimic (Figures 7(c) and 7(d)). These results indicated that miR-328-5p directly regulates WWP2. And miR-328-5p inhibitor significantly upregulated WWP2 and Bcl-2 gene expression, while Bax and Caspase3 was downregulated compared to the control group (Figures 7(e) and 7(f)). And WWP2 and Bcl-2 protein expressions were upregulated, while Bax and Caspase3 expressions were downregulated posttransfection of miR-328-5p inhibitor (Figures 7(g)–7(i)). Transfection of the WWP2 siRNA into the miR-328-5p inhibitor group reversed these effects (Figures 7(g)–7(i)). In conclusion, these results confirm that miR-328-5p promotes the apoptosis of NP cells by directly targeting WWP2.

4. Discussion

Studies found that miRNA is mainly involved in IDD by regulating cell apoptosis and proliferation [41–44], inflammatory reaction [45–47], and extracellular matrix component degradation [48–51]. Some studies have found miR-328-5p regulated the proliferation and apoptosis of cancer cells [29–32]. Cao et al. found that silencing miR-328-5p significantly inhibited the proliferation of non-small cell lung
cancer [29], and lncRNA RP5-916L7.2 inhibited miR-328-5p expression and promoted the apoptosis of tongue squamous cell carcinoma cells [30]. Luo et al. speculated that miR-328-5p was a tumor suppressor, and they confirmed that miR-328-5p mimic decreased obviously the proliferation and cell cycle of breast cancer cells, and promoted apoptosis [31]. Overexpression of LINC00210 significantly decreased miR-328-5p expression and increased the proliferation and migration of non-small cell lung cancer cells [32]. As we know, WWP2 is essential for maintaining a stable cell cycle, though silencing of WWP2 reduces the rate of proliferation, and WWP2 regulates various cellular processes such as protein degradation, membrane protein endocytosis, apoptosis, and gene transcription. [52]. WWP2 accelerates the cell cycle and promotes tumor formation [34]. Downregulation of WWP2 decreased obviously lung adenocarcinoma proliferation [37]. Xu et al. found that WWP2 siRNA inhibited Bcl-2 expression by promoting Bax and Caspase7/8 to induce apoptosis of liver cancer cells [53]. Even more, overexpression of WWP2 could inhibit the apoptosis of human renal tubular epithelial cells by inducing Bcl-2 expression and inhibiting Bax expression [40]. However, the regulatory mechanism of miR-328-5p and its target gene WWP2 in IDD has not been reported.

In the study, through the analysis of miRNA and mRNA microarray database, we screened the target genes miR-328-5p and WWP2. The high expression of miR-328-5p, while low expression of WWP2 in a degenerative tissues by qRT-PCR. Surprisingly, the expression of miR-328-5p was positively correlated, while that of WWP2 negatively correlated with the degeneration grade of IDD. And we also identified that Bax and Caspase3 expression were upregulated, while Bcl-2 expression is downregulated. After miR-328-5p mimic transfected into nucleus pulposus cell, we found that the expressions of WWP2 and Bcl-2 were downregulated, while the expressions of Bax and Caspase3 were upregulated, and the same results were obtained by knocking down WWP2., and WWP2 siRNA could significantly reverse the effect of miR-328-5p inhibitor. MiR-328-5p mimic significantly inhibited the proliferation of nucleus pulposus cells compared with the control group by CCK8 assay. We also confirmed that miR-328-5p mimic increaed obviously the apoptosis of nucleus pulposus cells. WWP2 was identified as the direct target gene of miR-328-5p by bioinformatics. Compared with WWP2 Mut group, the luciferase activity of nucleus pulposus cells in WWP2 WT group was significantly decreased, and WWP2 protein expression was also significantly downregulated.

In conclusion, these results strengthen our hypothesis that miR-328-5p regulated the prevalence and development of IDD by targeting WWP2. These results also indicate that miR-328-5p plays an essential role in regulating the proliferation and apoptosis of nucleus pulposus cells.

5. Conclusion

In conclusion, our results suggest that miR-328-5p is involved in the development of IDD by targeting WWP2 to induce the proliferation and apoptosis of nucleus pulpo-

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**Data Availability**

We confirm that this study data are available within the article or our supplementary materials. And the microarray data (such as Figure 1 and Figure 2) used in this study are available at the following link. The mRNA chip database is as follows:https://www.ncbi.nlm.nih.gov/gds/?term=GSE63492. The mRNA microarray database is as follows:https://www.ncbi.nlm.nih.gov/gds/?term=GSE34095.

**Conflicts of Interest**

The authors declare that there are no any competing financial interests.

**Authors’ Contributions**

Jing Yan, Lun-Gang Wu, and Ming Zhang contributed equally to this work.

**References**

[1] GBD 2017 Disease and injury incidence and prevalence collaborators, “Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the global burden of disease study 2017,” Lancet, vol. 392, 2018.

[2] L. Lao, M. D. Daubs, T. P. Scott et al., “Effect of disc degeneration on lumbar segmental mobility analyzed by kinetic magnetic resonance imaging,” Spine Phila Pa 2015, vol. 40, no. 5, pp. 316–322, 2015.

[3] D. Samartzis, J. Karppinen, F. Mok, D. Y. T. Fong, K. D. K. Luk, and K. M. C. Cheung, “A population-based study of juvenile disc degeneration and its association with overweight and obesity, low back pain, and diminished functional status,” The Journal of Bone and Joint Surgery. American Volume, vol. 93, no. 7, pp. 662–670, 2011.

[4] T. Ochi, Y. Taniguchi, Y. Oshima, S. Tanaka, and T. Saito, “Pathomechanism of intervertebral disc degeneration,” JOR Spine., vol. 3, no. 1, article e1076, 2020.

[5] H. R. Li, Q. Cui, Z. Y. Dong, J. H. Zhang, H. Q. Li, and L. Zhao, “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), vol. 41, no. 3, pp. E116–E123, 2016.

[6] J. C. Kennon, M. E. Awad, N. Chutkan, J. DeVine, and S. Fulzele, “Current insights on use of growth factors as therapy for intervertebral disc degeneration,” Biomolecular Concepts, vol. 9, no. 1, pp. 43–52, 2018.

[7] H. Chen, J. Wang, B. Hu et al., “MiR-34a promotes fas-mediated cartilage endplate chondrocyte apoptosis by targeting Bcl-2,” Molecular and Cellular Biochemistry, vol. 406, no. 1-2, pp. 21–30, 2015.

[8] C. L. Le Maitre, A. L. Binch, and A. A. Thorpe, “Degeneration of the intervertebral disc with new approaches for treating low back pain,” Journal of Neurosurgical Sciences, vol. 59, no. 1, pp. 47–61, 2015.
F. Ding, Z. W. Shao, and L. M. Xiong, "Matrix synthesis and degradation in human intervertebral disc degeneration," Biochemical Society Transactions, vol. 35, Part 4, pp. 652–655, 2007.

Z. Liu, C. Ma, J. I. Shen, D. Wang, J. Hao, and Z. Hu, "SDF-1/CXCR4 axis induces apoptosis of human degenerative nucleus pulposus cells via the NF-xB pathway," Molecular Medicine Reports, vol. 14, no. 1, pp. 783–789, 2016.

J. Shen, J. Fang, J. Hao et al., "The roles and perspectives of microRNAs as biomarkers for intervertebral disc degeneration," Journal of Tissue Engineering and Regenerative Medicine, vol. 11, no. 12, pp. 3481–3487, 2017.

F. Cao, Z. Wang, Y. Feng et al., "IncrRNA TTNTEP1 competitively sponges miR-328-5p to inhibit the proliferation of non-small cell lung cancer cells," Oncology Reports, vol. 43, no. 5, pp. 1606–1618, 2020.

Y. Chen, Y. Guo, and W. Yan, "IncrRNA RPS-916L7.2 correlates with advanced tumor stage, and promotes cells proliferation while inhibits cells apoptosis through targeting miR-328 and miR-939 in tongue squamous cell carcinoma," Clinical Biochemistry, vol. 67, pp. 24–32, 2019.

T. Luo, Y. Yan, Q. He, X. Ma, and W. Wang, "miR-328-5p inhibits MDA-MB-231 breast cancer cell proliferation by targeting RAGE," Oncology Reports, vol. 39, no. 6, pp. 2906–2914, 2018.

Z. Liu, L. Xu, K. Zhang, B. Guo, Z. Cui, and N. Gao, "LINC00210 plays oncogenic roles in non-small cell lung cancer by sponging microRNA-328-5p," Experimental and Therapeutic Medicine, vol. 19, no. 5, pp. 3325–3331, 2020.

A. E. Clements, V. Bravo, C. Koivisto, D. E. Cohn, and G. Leone, "WWP2 and its association with PTEN in endometrial cancer," Gynecol Oncol Rep., vol. 13, pp. 26–29, 2015.

C. Fukumoto, D. Nakashima, A. Kasamatsu et al., "WWP2 is overexpressed in human oral cancer, determining tumor size and poor prognosis in patients: downregulation of WWP2 inhibits the AKT signaling and tumor growth in mice," Oncoscience., vol. 1, no. 12, pp. 807–820, 2014.

J. Liang, W. F. Qi, S. Xie et al., "Expression of WW domain-containing protein 2 is correlated with pathological grade and recurrence of glioma," Journal of Cancer Research and Therapeutics, vol. 13, no. 6, pp. 1032–1037, 2017.

S. M. Soond, P. G. Smith, L. Wahl et al., "Novel WWP2 ubiquitin ligase isoforms as potential prognostic markers and molecular targets in cancer," Biochimica et Biophysica Acta, vol. 1832, no. 12, pp. 2127–2135, 2013.

Z. Wang, X. Zhang, C. Huang, and G. Zhang, "Elevated expression of WWP2 in human lung adenocarcinoma and its effect on migration and invasion," Biochemical and Biophysical Research Communications, vol. 479, no. 2, pp. 146–151, 2016.

J. G. Jung, A. Stocke, B. Guan et al., "Notch3 interactome analysis identified WWP2 as a negative regulator of notch3 signaling in ovarian cancer," PLoS Genetics, vol. 10, no. 10, article e1004751, 2014.

M. L. Ji, H. Jiang, X. J. Zhang et al., "Preclinical development of a microRNA-based therapy for intervertebral disc degeneration," Nature Communications, vol. 9, no. 1, p. 5051, 2018.
H. Che, W. He, J. Feng et al., “WWP2 ameliorates acute kidney injury by mediating p53 ubiquitylation and degradation,” Cell Biochemistry and Function, vol. 38, no. 6, pp. 695–701, 2020.

D.-Y. Zhang, Z.-J. Wang, Y.-B. Yu, Y. Zhang, and X.-X. Zhang, “Role of microRNA-210 in human intervertebral disc degeneration,” Experimental and Therapeutic Medicine, vol. 11, no. 6, pp. 2349–2354, 2016.

P. Liu, F. Chang, T. Zhang et al., “Downregulation of microRNA-125a is involved in intervertebral disc degeneration by targeting pro-apoptotic Bcl-2 antagonist killer 1,” Iranian Journal of Basic Medical Sciences, vol. 20, no. 11, pp. 1260–1267, 2017.

B. Wang, D. Wang, T. Yan, and H. Yuan, “MiR-138-5p promotes TNF-α-induced apoptosis in human intervertebral disc degeneration by targeting SIRT1 through PTEN/PI3K/Akt signaling,” Experimental Cell Research, vol. 345, no. 2, pp. 199–205, 2016.

P. Cai, T. Yang, X. Jiang, M. Zheng, G. Xu, and J. Xia, “Role of miR-15a in intervertebral disc degeneration through targeting MAP3K9,” Biomedicine & Pharmacotherapy, vol. 87, pp. 568–574, 2017.

K. Wang, T. Chen, X. Ying et al., “Ligustilide alleviated IL-1β induced apoptosis and extracellular matrix degradation of nucleus pulposus cells and attenuates intervertebral disc degeneration in vivo,” International Immunopharmacology, vol. 69, pp. 398–407, 2019.

Z. Liao, X. Wu, Y. Song et al., “Angiopoietin-like protein 8 expression and association with extracellular matrix metabolism and inflammation during intervertebral disc degeneration,” Journal of Cellular and Molecular Medicine, vol. 23, no. 8, pp. 5737–5750, 2019.

S. E. Navone, G. Marfia, A. Giannoni et al., “Inflammatory mediators and signalling pathways controlling intervertebral disc degeneration,” Histology and Histopathology, vol. 32, no. 6, pp. 523–542, 2017.

W. Liu, Y. Zhang, X. Feng et al., “Inhibition of microRNA-34a prevents IL-1β-induced extracellular matrix degradation in nucleus pulposus by increasing GDF5 expression,” Experimental Biology and Medicine (Maywood, N.J.), vol. 241, no. 17, pp. 1924–1932, 2016.

H. Tan, L. Zhao, R. Song, Y. Liu, and L. Wang, “microRNA-665 promotes the proliferation and matrix degradation of nucleus pulposus through targeting GDF5 in intervertebral disc degeneration,” Journal of Cellular Biochemistry, vol. 119, no. 9, pp. 7218–7225, 2018.

M. L. Ji, J. Lu, P. L. Shi et al., “Dysregulated miR-98 contributes to extracellular matrix degradation by targeting IL-6/STAT3 signaling pathway in human intervertebral disc degeneration,” Journal of Bone and Mineral Research, vol. 31, no. 4, pp. 900–909, 2016.

B. Zhang, W. Guo, C. Sun et al., “Dysregulated MiR-3150a-3p promotes lumbar intervertebral disc degeneration by targeting aggrecan,” Cellular Physiology and Biochemistry, vol. 45, no. 6, pp. 2506–2515, 2018.

B. H. Choi, X. Che, C. Chen, L. Lu, and W. Dai, “WWP2 is required for normal cell cycle progression,” Genes & Cancer, vol. 6, no. 9-10, pp. 371–377, 2015.

S.-Q. Xu, Y. Qin, D.-B. Pan et al., “Inhibition of WWP2 suppresses proliferation, and induces G1 cell cycle arrest and apoptosis in liver cancer cells,” Molecular Medicine Reports, vol. 13, no. 3, pp. 2261–2266, 2016.