IncRNA/circRNA-miRNA-mRNA ceRNA network in lumbar intervertebral disc degeneration

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Abstract. Accumulating evidence has indicated that noncoding RNAs are involved in intervertebral disc degeneration (IDD); however, the competing endogenous RNA (ceRNA)-mediated regulatory mechanisms in IDD remain rarely reported. The present study aimed to comprehensively investigate the alterations in expression levels of circular RNA (circRNA), long noncoding RNA (lncRNA), microRNA (miRNA/miR) and mRNA in the nucleus pulposus (NP) of patients with IDD. In addition, crucial lncRNA/circRNA-miRNA-mRNA ceRNA interaction axes were screened using the GSE67567 microarray dataset obtained from the Gene Expression Omnibus database. After data preprocessing, differentially expressed circRNAs (DEcs), lncRNAs (DEls), miRNAs (DEMs) or genes (DEGs) between IDD and normal controls were identified using the Linear Models for Microarray data method. A protein-protein interaction (PPI) network was constructed for DEGs based on protein databases, followed by module analysis. The ceRNA network was constructed based on the interaction between miRNAs and mRNAs, and lncRNAs/circRNAs and miRNAs. The underlying functions of mRNAs were predicted using the Database for Annotation, Visualization and Integrated Discovery database. The present study identified 636 DECs, 115 DEls, 84 DEMs and 1,040 DEGs between patients with IDD and control individuals. PPI network analysis demonstrated that Fos proto-oncogene, AP-1 transcription factor subunit (FOS), mitogen-activated protein kinase 1 (MAPK1), hypoxia inducible factor 1 (HIF1A) and transforming growth factor beta 1 (TGFB1) were hub genes and enriched in modules. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)/hsa_circRNA_102348-hsa-miR-185-5p-TGFB1/FOS, MALAT1-hsa-miR-155-5p-HIF1A, hsa_circRNA_102399-hsa-miR-302a-3p-HIF1A, MALAT1-hsa-miR-519d-3p-MAPK1 and hsa_circRNA_100086-hsa-miR-509-3p-MAPK1 ceRNA axes were obtained by constructing the ceRNA networks. In conclusion, these identified ceRNA interaction axes may be crucial targets for the treatment of IDD.

Introduction

Intervertebral disc degeneration (IDD) is characterized by excessive apoptosis of nucleus pulposus (NP) cells and degradation of extracellular matrix (ECM) components, and is considered the main contributing factor to lower back pain (LBP). It is estimated that ~84% of people will experience LBP during their lives worldwide, with 10% becoming chronically disabled (1), thus seriously affecting quality of life and imposing heavy economic burdens on families and society. Currently, clinical interventions for IDD primarily include conservative medication and surgery (spinal fusion or total disc replacement); however, these treatments are only able to temporarily relieve pain symptoms, without solving the underlying issues in IDD and providing a permanent cure (2,3). Therefore, it is necessary to deeply investigate the underlying mechanisms of IDD, in order to develop more effective strategies for preventing and treating IDD-associated LBP.

Recently, emerging evidence has suggested that noncoding RNAs, including microRNAs (miRNAs/miRs), long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) serve crucial roles in various biological processes, including cell proliferation and apoptosis (4). miRNAs function by binding to complementary sequences in the 3’-untranslated region (UTR) of their target mRNAs, thereby triggering either translational inhibition or mRNA degradation of the transcript (5). lncRNAs/circRNAs may act as competing endogenous RNAs (ceRNAs) by competitively binding to miRNAs through their miRNA response elements, thus regulating the expression levels of miRNA target mRNAs (6). Therefore, lncRNA/circRNA-miRNA-mRNA interactions may be an important mechanism underlying the initiation and development of IDD. This hypothesis has been verified in previous studies. Notably, Xi et al (7) demonstrated that IncRNA HLA complex group 18 (HCG18) is significantly upregulated in patients with IDD and its expression is positively correlated with disc degeneration grade. Subsequently, a luciferase reporter assay was conducted, which indicated...
that HCG18 may act as an endogenous sponge to downregulate miR-146a-5p expression in NP cells, thus promoting the upregulation of a miR-146a-5p target gene, TNF receptorsubassociated factor 6, ultimately suppressing the growth of NP cells by decreasing cell numbers in S phase of the cell cycle, inducing cell apoptosis, recruiting macrophages and hypercalcification (7). Wang et al (8) demonstrated that IncRNA RP11-296A18.3 interacts with miR-138 to induce upregulation of the miR-138 target gene, hypoxia inducible factor 1 subunit α (HIF1A), thus affecting NP proliferation and ECM synthesis. The expression of RP11-296A18.3 is positively correlated with HIF1A; however, RP11-296A18.3 and HIF1A are inversely correlated with miR-138 in IDD tissues (8). In addition, by circRNA microarray assay, bioinformatics analysis, RNA immunoprecipitation and luciferase assay, Wang et al (9) provided evidence to suggest that circRNA-4099 is able to function as a ‘sponge’ by competitively binding miR-616-5p, which reverses the suppression of SRY-box 9 by miR-616-5p. Cheng et al (10) revealed that circVMA21 acts as a sponge of miR-200c, thus regulating the expression of the target mRNA, X-linked inhibitor of apoptosis (XIAP). The decreased expression of XIAP in inflammatory cytokines-treated NP cells and degenerative NP tissues is directly associated with excessive apoptosis, and an imbalance between the anabolic and catabolic factors of ECM. Conversely, intradiscal injection of circVMA21 may alleviate IDD in a rat model (10). These findings indicated that targeting the regulatory effects of associated lncRNAs, circRNAs, miRNAs and mRNAs may have a potential role in the clinical treatment of IDD. However, to the best of our knowledge, IDD-associated lncRNA/circRNA-miRNA-mRNA ceRNA regulatory mechanisms remain rarely reported, until now (11).

The present study aimed to preliminarily identify novel lncRNA/circRNA-miRNA-mRNA ceRNA-mediated regulatory mechanisms in IDD by constructing a ceRNA regulatory network using microarray data collected from a public database. This study may provide targets for the development of novel therapeutic strategies to treat IDD.

Materials and methods

Gene expression omnibus (GEO) dataset collection. A microarray dataset was retrieved from the public GEO database (www.ncbi.nlm.nih.gov/geo); the accession number of the dataset used is GSE67567, which contains three sub-datasets: GSE67566, circRNA expression profile (platform: GPL19978, Agilent-069978 Arraystar Human CircRNA microarray V1; Agilent Technologies, Inc., Santa Clara, CA, USA) (12,13); GSE63492, miRNA expression profile [platform: GPL19449, Exiqon miRCURY LNA microRNA Array, 7th generation REV-hsa, mmu & rno (miBase v18.0); Exiqon; Qiagen, Inc., Valencia, CA, USA] (12,13); and GSE56081, mRNA-lncRNA expression profile [platform: GPL15314, Arraystar Human LncRNA microarray V2.0 (Agilent_033010 Probe Name version); Agilent Technologies, Inc.] (12,14). These three sub-datasets included NP samples derived from five normal control individuals and five patients with IDD.

Data preprocessing and differential expression analysis. The raw TXT data collected from the microarray platforms were preprocessed using the Linear Models for Microarray data (LIMMA) method (15) (version 3.34.0; www.bioconductor.org/packages/release/bioc/html/limma.html) in the Bioconductor R package (version 3.4.1; www.R-project.org), including base-2 logarithmic (log2) transformation and quantile normalization. For the GSE56081 microarray data, the probe sequences were downloaded from the annotation platforms and aligned with the human genome using Clustal W computer program (version 2; www.clustal.org) to obtain the expression levels of lncRNA and mRNAs.

The lists of differentially expressed genes (DEGs), differentially expressed lncRNAs (DEls), differentially expressed circRNAs (DECs) and differentially expressed miRNAs (DEMs) between controls and patients with IDD were generated using the LIMMA method (15), where statistical significance was set as log-fold change (logFC)>1 and Benjamini and Hochberg-corrected (16) false discovery rates (FDR)<0.05. A hierarchical cluster heatmap representing expression intensity and direction was created using pheatmap in R package (version: 1.0.8; cran.r-project.org/web/packages/pheatmap) based on Euclidean distance.

miRNA regulatory network construction. The DEM-associated target genes were predicted using the starBase database (version 2.0; starbase.sysu.edu.cn/index.php) (21) which provides the prediction results of five miRNA databases (TargetScan, picTar, RNA22, PITA and miRanda). The miRNA-target gene interaction pairs were selected if they were predicted in ≥1 database. The target genes were then overlapped with the DEGs, and the negative interaction pairs between DEMs and DEGs (according to their expression levels) were used to construct the miRNA-mRNA network using Cytoscape software (version 3.6.1; www.cytoscape.org) (17). The known miRNAs associated with IDD were predicted using the Human microRNA Disease Database (HMDD; www.cuilab.cn/hmdd) (22).

CeRNA regulatory network construction. The starBase database (version 2.0; starbase.sysu.edu.cn/index.php) (21) database was used to screen the interactions between DEls and DEMs,
which were then integrated with the miRNA-mRNA interactions to establish the DEL-DEM-DEG ceRNA network using Cytoscape software (version 3.6.1; www.cytoscape.org) (17).

Human sequences of DEMs and DECs were downloaded from the circBase (www.circbase.org) (23) and miRBase (version 21; www.mirbase.org) (24) databases, respectively. mirVana (cbio.mskcc.org/mirna2003/miranda.html) (25) was used to predict the interactions between DECs and DEMs according to the following parameter settings: Gap Open Penalty, -8; Gap Extend, -2; Score Threshold, 80%; and Energy Threshold, -20. The interaction pairs between DECs and DEMs were then integrated with the miRNA-mRNA interactions to establish the DEC-DEM-DEG ceRNA network using Cytoscape software (version 3.6.1; www.cytoscape.org) (17).

The overlapped miRNA-mRNAs in the above two ceRNA networks were also selected to construct the lncRNA/circRNA-miRNA-mRNA network.

**Functional enrichment analysis.** The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of genes in each module and network was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool (version 6.8; http://david.abcc.ncifcrf.gov) (26), with P<0.05 set as the cut-off value. In addition, all known IDD-associated pathways were downloaded from the Comparative Toxigenomics Database (CTD; ctd.mdiblr.org) (27), which were then overlapped with the enriched ceRNA pathways, in order to obtain an IDD pathway-associated ceRNA network.

**Results**

**Differential expression analysis.** According to the pre-set threshold (FDR<0.05 and \( \log_{2} \text{FC}>1 \)), a total of 636 DECs were identified between IDD and control samples, including 354 upregulated and 282 downregulated circRNAs; 115 DELs were screened, consisting of 50 upregulated and 65 downregulated lncRNAs; 84 DEMs were obtained, containing 50 upregulated and 34 downregulated miRNAs; and 1,040 DEGs were generated, comprising 763 upregulated and 277 downregulated genes. The top 20 DECs, DELs, DEMs and DEGs are presented in Table I. The hierarchical cluster heat maps indicated that these DECs (Fig. 1A), DELs (Fig. 1B), DEMs (Fig. 1C) and DEGs (Fig. 1D) could distinguish IDD from control samples.

**PPI network.** A PPI network was constructed using the screened DEGs, which included 721 nodes (561 upregulated and 160 downregulated) and 3,561 interaction pairs. DNA topoisomerase II \( \beta \), matrix metallopeptidase 2 (MMP2), enolase (ENO1), Fos proto-oncogene, AP-1 transcription factor subunit (FOS), mitogen-activated protein kinase 1 (MAPK1), HIF1A, protein phosphatase 2 scaffold subunit Act (PPP2R1A), ENO2, RNA polymerase II subunit C (POLR2C), transforming growth factor \( \beta \) (TGFBI), fibronectin 1 (FN1), Jun proto-oncogene, AP-1 transcription factor subunit (JUN), vimentin and H2A histone family member V (H2AFV) were considered hub genes in the PPI network because they were present in the top 35 genes of four topological features (Tables II and III). Six functionally related and highly interconnected modules were subsequently extracted using MCODE, in order to further screen crucial genes (Fig. 2). Among them, the hub genes PPP2R1A, TGFBI, POLR2C and FN1 were included in module 2; H2AFV was included in module 4; FOS was included in module 5; and MMP2 and MAPK1 were contained in module 6.

The genes in each module were then subjected to analysis using DAVID, in order to predict their functions. The results of KEGG enrichment analysis (Table IV) demonstrated that in module 2, PPP2R1A was enriched in ‘mRNA surveillance pathway’ and TGFBI was enriched in ‘Cytokine-cytokine receptor interaction’; in module 4, H2AFV was enriched in ‘Systemic lupus erythematosus’; in module 5, FOS was involved in ‘Rheumatoid arthritis’ and ‘Human T-cell leukemia virus 1 infection’; and in module 6, MAPK1 and MMP2 participated in ‘Estrogen signaling pathway’. The other hub genes were not enriched in KEGG pathways.

**miRNA regulatory network.** A total of 305 differentially expressed target genes were predicted for 14 of the 84 DEMs, which were used to construct the miRNA-mRNA network (Fig. 3). This network included 522 negative interaction relationships between 14 DEMs and 305 DEGs (six upregulated DEMs regulated 45 downregulated DEGs; eight downregulated DEMs regulated 260 upregulated DEGs, such as hsa-miR-155-5p/hsa-miR-302a-3p/hsa-miR-519d-3p-HIF1A, hsa-miR-185-5p-TGFBI,hsa-miR-185-5p/hsa-miR-155-5p-FOS and hsa-miR-509-3p/hsa-miR-519d-3p-MAPK1). Among all of the DEMs in this miRNA regulatory network, hsa-miR-155-5p was revealed to be associated with IDD, as determined by searching the HMDD database.

The underlying functions of the DEGs in the miRNA-mRNA network were also analyzed by DAVID database. The results indicated that ‘TGF-beta signaling pathway’ (MAPK1 and TGFBI), ‘Adherens junction’ (MAPK1), ‘MAPK signaling pathway’ (MAPK1, TGFBI and FOS), ‘Cell cycle’ (TGFBI), ‘mTOR signaling pathway’ (MAPK1 and HIF1A), ‘Toll-like receptor signaling pathway’ (MAPK1 and FOS), ‘B cell receptor signaling pathway’ (MAPK1 and FOS) and ‘T cell receptor signaling pathway’ (MAPK1 and FOS) pathways were significantly enriched for genes in the miRNA-mRNA network (Table V).

**CeRNA network.** Using the starBase database, nine DEMs were predicted to regulate 12 DELs; this was used to establish the lncRNA-miRNA-mRNA ceRNA network via integration with the miRNA-mRNA network (Fig. 4). This network comprised 280 nodes (nine DEMs; 12 DELs; 259 DEGs) and 407 interactions (16 DEL-DEM and 391 DEM-DEG interactions). Notably, upregulated metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) may function as a ceRNA to suppress the inhibitory effects of hsa-miR-155-5p on HIF1A and FOS, thus leading to their upregulated expression. Similarly, upregulated MALAT1 may regulate the targeted effects of hsa-miR-185-5p on TGFBI and FOS, as well as hsa-miR-519d-3p on HIF1A and MAPK1. The functional analysis of genes in the lncRNA-associated ceRNA network were significantly enriched in ‘TGF-beta signaling pathway’, ‘FoxO signaling pathway’ and ‘TNF signaling pathway’ (Table V).
Table I. Top upregulated and downregulated differentially expressed circRNAs, lncRNAs, miRNAs and mRNAs identified from Gene Expression Omnibus microarray datasets.

### A. circRNAs

| RNA                | logFC | FDR       |
|--------------------|-------|-----------|
| hsa_circRNA_101852 | 2.98  | 3.92×10⁻¹⁵|
| hsa_circRNA_101853 | 2.93  | 6.98×10⁻¹⁶|
| hsa_circRNA_101139 | 2.92  | 6.98×10⁻¹⁶|
| hsa_circRNA_103890 | 2.86  | 1.72×10⁻¹⁵|
| hsa_circRNA_400019 | 2.84  | 3.87×10⁻¹⁴|
| hsa_circRNA_102324 | 2.78  | 1.00×10⁻¹⁵|
| hsa_circRNA_104703 | 2.72  | 1.24×10⁻¹⁵|
| hsa_circRNA_104600 | 2.68  | 7.51×10⁻¹⁵|
| hsa_circRNA_100604 | 2.68  | 1.57×10⁻¹⁵|
| hsa_circRNA_100018 | 2.61  | 1.67×10⁻¹⁵|
| hsa_circRNA_103410 | 2.59  | 6.98×10⁻¹⁶|
| hsa_circRNA_000200 | 2.56  | 2.32×10⁻¹⁴|
| hsa_circRNA_100086 | 2.32  | 7.03×10⁻¹⁴|
| hsa_circRNA_102348 | 1.95  | 9.57×10⁻¹⁴|
| hsa_circRNA_102399 | 1.63  | 4.92×10⁻¹²|
| hsa_circRNA_101645 | -3.30 | 1.60×10⁻¹⁴|
| hsa_circRNA_104508 | -3.26 | 2.19×10⁻¹³|
| hsa_circRNA_102116 | -3.18 | 1.92×10⁻¹⁴|
| hsa_circRNA_103838 | -3.06 | 6.98×10⁻¹⁶|
| hsa_circRNA_101557 | -3.05 | 1.96×10⁻¹⁴|

### B. lncRNAs

| RNA        | logFC | FDR       |
|------------|-------|-----------|
| TRPC7-AS1  | 6.61  | 6.55×10⁻⁸ |
| MIR4458HG  | 1.40  | 5.56×10⁻³ |
| GAS5       | 1.40  | 4.30×10⁻² |
| CBR3-AS1   | 1.40  | 3.05×10⁻⁴ |
| ADPGK-AS1  | 1.40  | 2.67×10⁻³ |
| SNHG5      | 1.40  | 2.67×10⁻² |
| ADARB2-AS1 | 1.391 | 4.17×10⁻⁵ |
| LINC00431  | 1.39  | 4.68×10⁻⁸ |
| MCCC1-AS1  | 1.39  | 8.89×10⁻⁵ |
| MALAT1     | 1.07  | 6.58×10⁻⁴ |
| HOTAIR     | -7.21 | 6.47×10⁻⁸ |
| LINC0957   | -6.28 | 7.33×10⁻⁹ |
| VPS13A-AS1 | -6.01 | 1.81×10⁻⁸ |
| IL10B-AS1  | -5.54 | 9.48×10⁻⁹ |
| MAPT-AS1   | -5.12 | 6.53×10⁻⁹ |
| LINC00689  | -3.63 | 2.87×10⁻⁵ |
| EFCAB6-AS1 | -3.39 | 2.96×10⁻⁸ |
| HAND2-AS1  | -3.21 | 3.47×10⁻⁴ |
| LINC00884  | -3.12 | 5.11×10⁻⁵ |
| LINC01405  | -3.04 | 9.52×10⁻⁴ |

### C. miRNAs

| RNA       | logFC | FDR       |
|-----------|-------|-----------|
| hsa-miR-4287 | 5.81  | 4.50×10⁻³ |

### D. mRNAs

| RNA        | logFC | FDR       |
|------------|-------|-----------|
| HBB        | 8.41  | 3.87×10⁻⁸ |
| HBA1       | 8.19  | 8.62×10⁻¹¹|
| COL1A2     | 7.01  | 3.41×10⁻⁷ |
| PTP4A3     | 6.98  | 7.31×10⁻⁷ |
| RBM38      | 6.70  | 1.72×10⁻⁹ |
| MFA04      | 6.19  | 7.41×10⁻⁹ |
| GREM1      | 5.77  | 4.49×10⁻⁸ |
| NKG7       | 5.55  | 1.13×10⁻⁹ |
| TREM1      | 5.47  | 1.12×10⁻⁹ |
| LUM        | 5.36  | 3.28×10⁻⁸ |
| PHLD2      | -7.09 | 2.99×10⁻⁴ |
| KIAA0319   | -6.95 | 5.16×10⁻¹⁰|
| ERLIN1     | -6.89 | 6.61×10⁻⁹ |
| APOD       | -6.47 | 4.61×10⁻⁷ |
| SLF2       | -6.45 | 5.61×10⁻¹⁰|
| NDRG4      | -6.44 | 4.91×10⁻¹⁰|
| GUCY1A3    | -6.43 | 9.45×10⁻¹⁰|
| PLAG1      | -6.41 | 1.67×10⁻⁷ |
| ATP8B3     | -6.32 | 7.34×10⁻⁹ |

Positive logFC values indicate upregulated expression in IDD samples compared with in control samples, whereas negative logFC values indicate downregulated expression in IDD samples compared with in control samples. All microarray datasets were retrieved from public Gene Expression Omnibus databases, including GSE67566 (12,13) for circRNA expression; GSE63492 (12,13) for miRNA expression and GSE56081 (12,14) for mRNA-lncRNA expression. circRNA, circular RNA; FC, fold change; FDR, false discovery rates; IDD, intervertebral disc degeneration; lncRNA, long noncoding RNA; mRNA, microRNA.
MAP1K, FOS and TGFB1 were each included in at least one of these pathways.

Using the miRanda database, 61 DEMs were predicted to regulate 63 decs; this information was used to establish the circRNA-miRNA-mRNA cerna network via integration with the miRNA-mRNA network (Fig. 5). Notably, upregulated hsa_circRNA_102348 may function as a cerna to suppress the inhibitory effects of hsa-miR-185-5p on TGFB1 and FOS, thus resulting in their upregulated expression; upregulated hsa_circRNA_102399 may act as a cerna to regulate the hsa-mir-302a-3p-HiF1a interaction; hsa_circRNA_100086 may influence the regulatory effect of hsa-mir-509-3p on MaPK1. hsa_circRNA_102348 was selected because it has previously been studied in other diseases (28). In addition, hsa_circRNA_102399 (score threshold, 92%) and hsa_circRNA_100086 (score threshold, 90%) may be important in IDD, because they interacted with their miRNAs with the highest score thresholds and the expression trend of these circRNAs was opposite to that of their target miRNAs. Functional analysis of genes in the circRNA-related cerna network revealed that they were significantly enriched in ‘TGF-beta signaling pathway’ and ‘MAPK signaling pathway’;

MAP1K, TGFB1 and FOS were each included in at least one of these pathways (Table V).

Through analysis of the IncRNA and circRNA cerna networks, it was revealed that hsa-miR-185-5p, hsa-miR-486-5p, hsa-miR-196b-5p, hsa-miR-382-5p and hsa-miR-324-5p were included in both; therefore, an IncRNA/circRNA-miRNA-mRNA integrated network was also established (Fig. 6), in which hsa-miR-185-5p-TGFB1-associated cerna axes may be particularly important because TGFB1 was enriched in most of the KEGG pathways for this network (Table V).

**IDD pathway-related cerna network.** Using ‘intervertebral disc degeneration’ as the key search term, 30 KEGG pathways were screened from the CTD database; four pathways were overlapped with those enriched for genes in the integrated cerna network, including ‘TGF-beta signaling pathway’, ‘FoxO signaling pathway’, ‘Endocytosis’ and ‘Cell cycle’. The pathway-related genes were extracted to construct the IDD pathway-related cerna network (Fig. 7), in which the hsa_circRNA_102348/MalaT1-hsa-mir-185-5p-TGFB1 cerna axes were contained.

**Discussion**

The present study identified FOS, MAP1K, HiF1A and TGFB1 as crucial genes in IDD, as determined by topological feature analysis of genes in a PPI network and module screening. Subsequently, by constructing a cerna network, it was suggested that the upregulated IncRNA MalaT1 may be particularly important for IDD, as it may function as a cerna for downregulating hsa-miR-155-5p, hsa-miR-185-5p and hsa-miR-519d-3p expression, thus leading to the upregulation of FOS, TGFB1, HiF1A and MAP1K, respectively. In addition, upregulated hsa_circRNA_102348, hsa_circRNA_102399 and hsa_circRNA_100086 may function as ceRNAs to block the inhibitory effects of hsa-miR-185-5p, hsa-miR-302a-3p and hsa-miR-509-3p on the expression levels of TGFB1/FOS, HiF1A and MAP1K, respectively. MAP1K and FOS may be involved in IDD by influencing the MAPK pathway, thus affecting inflammatory pathways. KEGG analysis suggested that TGFB1 participated in IDD by affecting ‘TGF-beta signaling pathway’, ‘Cell cycle’ and ‘Cytokine-cytokine interaction’, whereas HiF1A may be associated with ‘mTOR signaling pathway’.

It has been reported that TGFB promotes NP cell proliferation and stimulates ECM synthesis (11). Therefore, overexpression of TGFB may have the potential to inhibit IDD and exert therapeutic effects; this has been demonstrated in several *in vivo* studies (29,30). Further studies have indicated that TGFB exerts anabolic effects on intervertebral discs by antagonizing inflammation (31,32). Furthermore, Zhang *et al* (33) reported that the expression levels of TGFB1 and C-C motif chemokine ligand (CCL)3/4 are elevated in degenerative NP tissue. TGFB1 treatment significantly inhibits CCL4 expression and prevents pain development in a rat model of IDD; this effect is dependent on the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway. Yang *et al* (34,35) observed that the inflammatory cytokine tumor necrosis factor (TNF)-α induces high expression of syndecan-4 in NP cells, which is required for MMP3 activation to trigger the progression of...
Table II. Topological features of DEGs in the protein-protein interaction network.

### A, Degree

| DEG  | Value |
|------|-------|
| MAPK1| 86    |
| JUN  | 78    |
| TOP2B| 75    |
| TGFB1| 70    |
| FOS  | 58    |
| BTRC | 49    |
| FN1  | 49    |
| H2AFV| 44    |
| SOCS3| 43    |
| POLR2C| 43   |
| UBE2C| 43    |
| CDC23| 42    |
| VIM  | 42    |
| KEAP1| 41    |
| MMP2 | 40    |
| ANAPC1| 40   |
| SKP2 | 40    |
| HIST2H3A| 40 |
| SMAD3| 39    |
| ENO2 | 39    |
| SMAD2| 38    |
| CDC26| 38    |
| UBE2G1| 37   |
| H2AFX| 37    |
| CSF2 | 37    |
| ENO1 | 37    |
| HIST2H2AA3| 36 |
| UBE2R2| 36   |
| HIST1H2BK| 36 |
| PPP2R1A| 36  |
| SIAH1| 36    |
| RPSA | 36    |
| FAU  | 35    |
| HERC3| 34    |
| HIF1A| 33    |

### B, CC

| DEG  | Value |
|------|-------|
| BRD9 | 1.0000|
| ST6GALNAC4| 0.6667 |
| SLC25A43| 0.6667 |
| ORMDL1| 0.6667 |
| GALNT1| 0.6667 |
| JUN  | 0.4257|
| MAPK1| 0.4250|
| FOS  | 0.4001|
| TGFB1| 0.3967|
| TOP2B| 0.3917|
| VIM  | 0.3842|
| FN1  | 0.3766|
| MMP2 | 0.3762|
| POLR2C| 0.3760|
| SMAD3| 0.3752|
| ENO1 | 0.3726|
| HIF1A| 0.3726|
| ACTL6A| 0.3720|
| HBA1 | 0.3714|
| ENO2 | 0.3707|
| H2AFV| 0.3674|
| H2AFX| 0.3674|
| SMAD2| 0.3668|
| PPP2R1A| 0.3651|

### C, BC

| DEG  | Value |
|------|-------|
| ST6GALNAC1| 1.0000|
| ZDHHC9| 1.0000|
| MAPK1| 0.1255|
| TOP2B| 0.0988|
| JUN  | 0.0913|
| TGFB1| 0.0619|
| FOS  | 0.0450|
| NME2 | 0.0413|
| FN1  | 0.0365|
| PPP2R1A| 0.0319|
| VIM  | 0.0314|
| POLR2C| 0.0310|
| RAD51| 0.0301|
| HIF1A| 0.0293|
| CTS2D| 0.0272|
| ENO2 | 0.0266|
| ACTG2| 0.0253|
| ENO1 | 0.0242|
| NDUF4A| 0.0239|
| H2AFV| 0.0227|
| MMP2 | 0.0224|
idd. TGFB1 suppresses TNF-α-mediated upregulation of syndecan-4 and MMP3 via ERK1/2 signaling pathways. In the present study, TGFB1 was significantly upregulated in patients with idd compared with in control individuals. This may be representative of the inflammatory activity present in IDD, and TGFB1 expression may be increased as an inflammatory stress response to antagonize inflammation (36); this was also indirectly reflected by the lower expression of several TGFB1-associated inflammatory genes, including C-X-C motif chemokine ligand (CXCL)1 and CXCL2 in this study. However, to the best of our knowledge, the regulatory mechanisms of TGFB1 in idd have not been explored.

The present study revealed that hsa-miR-185-5p may regulate the expression of TGFB1, whereas MALAT1 and
hsa_circRNA_102348 may function as ceRNAs to interact with hsa-miR-185-5p. To the best of our knowledge, no previous studies have investigated the roles of hsa-miR-185-5p, MALAT1 and hsa_circRNA_102348 in IDD. However, their known mechanisms in regulating cell proliferation in other diseases may indirectly explain their roles in IDD. Notably, Cheng et al. (37) demonstrated that miR-185 inhibits cell proliferation, while promoting apoptosis and autophagy through negative regulation of TGFBI in nasopharyngeal carcinoma. miR-185 mimics have been observed to exert inhibitory effects on osteoblasts through downregulating the Wnt/β-catenin axis (38). Li et al. (39) demonstrated that MALAT1 promotes proliferation of cardiac progenitor cells under hypoxic conditions and alleviates myocardial infarction-induced heart failure. Furthermore, Li et al. (40) revealed that MALAT1 promotes osteosarcoma cell growth and predicts an unfavorable outcome. Hereby, it was hypothesized that miR-185 may be downregulated and MALAT1 may be upregulated in IDD; this hypothesis was confirmed by the present results. Although, to the best of our knowledge, no experimental study has been conducted to validate the roles of hsa_circRNA_102348, its expression has been demonstrated to be upregulated in idiopathic pulmonary fibrosis (28); this is characterized by the abnormal deposition of ECM proteins. Therefore, the expression of hsa_circRNA_102348 may also be upregulated in IDD.

Hypoxia has been reported to promote disc cell proliferation (41). HIF1A is an important response gene under hypoxic and inflammatory conditions, which is upregulated in NP cells (42). Overexpression of HIF1A has been demonstrated to promote NP cell proliferation and lead to increased expression levels of collagen II and aggrecan in human NP cells (8,43). Therefore, upregulation of HIF1A may be a protective strategy to antagonize degenerative intervertebral discs (44). In line with Wang et al. (8), which reported that HIF1A was highly expressed in IDD and may exert a protective response against IDD, the mRNA expression of HIF1A was significantly upregulated in patients with IDD compared with in control individuals in the present study. However, whether HIF1A also causes the damage

Figure 2. Modules extracted from the protein-protein interaction network. (A) Module 1; (B) module 2; (C) module 3; (D) module 4; (E) module 5; and (F) module 6. Red represents upregulated expression, whereas green represents downregulated expression. The larger the size of the node (protein), the higher its degree (number of interactions). Hub genes are indicated by red boxes.
| Module | Term                                                                 | P-value       | Genes                                                                                           |
|--------|----------------------------------------------------------------------|---------------|-------------------------------------------------------------------------------------------------|
| A      | hsa04120: Ubiquitin mediated proteolysis                              | 1.09x10^{-23} | ANAPC1, SOCS3, BTRC, UBE2G1, FBXO2, SKP2, CDC23, HERC3, KEAP1, UBE2C, CDC26, UBE2R2, FBXW8, CUL7, SIAH1 |
|        | hsa04114: Oocyte meiosis                                              | 1.51x10^{-3}  | ANAPC1, BTRC, CDC23, CDC26                                                                    |
|        | hsa04110: Cell cycle                                                  | 2.19x10^{-3}  | ANAPC1, SKP2, CDC23, CDC26                                                                    |
|        | hsa04914: Progesterone-mediated oocyte maturation                     | 1.48x10^{-2}  | ANAPC1, CDC23, CDC26                                                                            |
| B      | hsa03010: Ribosome                                                    | 1.39x10^{-9}  | RPSA, RPS27, RPS29, RPL41, RPL13, RPL31, RPL15, FAU, RPL36, RPS15A, RPS20                       |
|        | hsa04062: Chemokine signaling pathway                                 | 4.75x10^{-5}  | CXCL1, CCR7, PPBP, ADCY7, CXCL2, PF4, CCL5, GNG4                                               |
|        | hsa03040: Spliceosome                                                | 4.70x10^{-5}  | BCA5S2, HNRNPK, SRSF6, PCBP1, HNRNP            |
|        | hsa04060: Cytokine-cytokine receptor interaction                      | 7.15x10^{-3}  | VEGFC, CCR7, PPBP, PF4, CCL5, TGFβ1                                                        |
| C      | hsa04974: Protein digestion and absorption                            | 6.15x10^{-9}  | COL4A3, COL9A3, COL4A1, COL1A2, COL2A1, COL5A3                                               |
|        | hsa04512: ECM-receptor interaction                                    | 7.97x10^{-7}  | COL4A3, COL4A1, COL1A2, COL2A1, COL5A3                                                       |
|        | hsa05146: Amoebiasis                                                 | 1.77x10^{-9}  | COL4A3, COL4A1, COL1A2, COL2A1, COL5A3                                                       |
|        | hsa04510: Focal adhesion                                             | 2.50x10^{-5}  | COL4A3, COL4A1, COL1A2, COL2A1, COL5A3                                                       |
|        | hsa04151: PI3K-Akt signaling pathway                                  | 1.90x10^{-4}  | COL4A3, COL4A1, COL1A2, COL2A1, COL5A3                                                       |
|        | hsa04611: Platelet activation                                        | 6.93x10^{-3}  | COL1A2, COL2A1, COL5A3                                                                         |
| D      | hsa05322: Systemic lupus erythematosus                               | 1.58x10^{-5}  | HIST2H3A, HIST2H2AA3, H2AFV, HIST1H2BK, H2AFX                                               |
|        | hsa05034: Alcoholism                                                 | 4.74x10^{-5}  | HIST2H3A, HIST2H2AA3, H2AFV, HIST1H2BK, H2AFX                                               |
| E      | hsa05323: Rheumatoid arthritis                                       | 5.45x10^{-3}  | CSF2, FOS, TNFSF11                                                                             |
|        | hsa05200: Pathways in cancer                                         | 1.19x10^{-3}  | FOS, FOXO1, FGFI3, SMAD2                                                                      |
|        | hsa04068: FoxO signaling pathway                                     | 1.23x10^{-2}  | FOXO1, SMAD2, IL7R                                                                            |
|        | hsa05202: Transcriptional misregulation in cancer                    | 1.89x10^{-2}  | CSF2, FOXO1, GZMB                                                                             |
|        | hsa04060: Cytokine-cytokine receptor interaction                      | 3.40x10^{-2}  | CSF2, TNFSF11, IL7R                                                                           |
|        | hsa05166: Human T-cell leukemia virus 1 infection                    | 4.14x10^{-2}  | CSF2, FOS, SMAD2                                                                              |
| F      | hsa04915: Estrogen signaling pathway                                 | 1.68x10^{-3}  | MAPK1, SHC1, HSPA1B, MMP2                                                                     |
observed in IDD remains unclear and requires further investigation. Furthermore, the present study demonstrated that hsa-miR-155-5p and hsa-miR-302a-3p could regulate HIF1a expression. MALAT1 could sponge hsa-miR-155-5p, whereas hsa_circRNA_102399 could sponge hsa-miR-302a-3p. Although their interaction mechanisms have not been analyzed in IDD, the roles of some miRNAs and lncRNAs have been confirmed in IDD and other diseases. For example, Wang et al. (45) and Ye et al. (46) demonstrated that mir-155 is downregulated in degenerative NP by microarray analysis; this was confirmed by reverse transcription-quantitative polymerase chain reaction. Zhang et al. (47) demonstrated that miR-155 is downregulated in degenerative NP by microarray analysis; this was confirmed by reverse transcription-quantitative polymerase chain reaction. Using gain- and loss-of-function experiments, miR-155 downregulation has been revealed to be mediated by MALAT1 (48), whereas miR-155 has also been demonstrated to directly target and silence HIF1A (49).

FOS is a member of the AP-1 transcription factor family, which has been demonstrated to be associated with numerous cellular processes, such as inhibition of chondrocyte differentiation (50), whereas chondrocyte transplantation has been proved to be a feasible and biologically relevant technique to repair disc damage and retard disc degeneration (51,52). Therefore, it was hypothesized that upregulated expression of FOS may be a risk factor that promotes the development of IDD. This hypothesis has been confirmed by a recent study, which used the AP-1 selective inhibitor T-5224 to demonstrate that inhibition of c-Fos/AP-1 prevents disc degeneration and associated pain (53). As an inflammatory response gene (54), FOS was revealed to be upregulated in this study. Similar to TGFβ1, hsa-miR-185-5p was shown to regulate the expression of

| Term                                | P-value | Genes                        |
|-------------------------------------|---------|-------------------------------|
| hsa04510: Focal adhesion            | 1.30x10⁻² | MAPK1, PGF, FLT4, SHC1         |
| hsa04015: Rap1 signaling pathway    | 1.37x10⁻² | MAPK1, PGF, FLT4, ITGAM       |
| hsa04014: Ras signaling pathway     | 1.67x10⁻² | MAPK1, PGF, FLT4, SHC1         |

Table I. IV. Continued.

| F, Module 6  |
|-------------|
| Term                                | P-value | Genes                        |
| hsa04510: Focal adhesion            | 1.30x10⁻² | MAPK1, PGF, FLT4, SHC1         |
| hsa04015: Rap1 signaling pathway    | 1.37x10⁻² | MAPK1, PGF, FLT4, ITGAM       |
| hsa04014: Ras signaling pathway     | 1.67x10⁻² | MAPK1, PGF, FLT4, SHC1         |

KEGG pathways were predicted using the Database for Annotation, Visualization and Integrated Discovery online tool (version 6.8; david.abcc.ncifcrf.gov) (26). KEGG, Kyoto Encyclopedia of Genes and Genomes.
Table V. KEGG pathway analysis of genes in regulatory networks.

A, miRNA-mRNA network

| Term                                      | P-value   | Genes                                      |
|-------------------------------------------|-----------|--------------------------------------------|
| hsa04350: TGF-beta signaling pathway      | 2.02x10^{-4} | MAPK1, ACVR1B, LTBP1, FST, TGFBR2, SMAD2, TGFB1 |
| hsa04120: Ubiquitin mediated proteolysis  | 1.80x10^{-3} | WWP2, SOCS3, UBE2G1, CDC23, SIAH1, HERC3, UBE2R |
| hsa04520: Adherens junction               | 2.98x10^{-3} | MAPK1, ACVR1B, TGFBR2, SMAD2, SNAI1       |
| hsa04010: MAPK signaling pathway          | 5.07x10^{-3} | MAPK1, FOS, ACVR1B, TGFBR2, MKNK2, FGF13, AKT3, TGFB1, ATFB |
| hsa04115: p53 signaling pathway           | 8.57x10^{-3} | CCND2, SIAH1, MDM4, SESN3                   |
| hsa00051: Fructose and mannose metabolism| 9.51x10^{-3} | PFKFB2, AKR1B1, PMM1                        |
| hsa04110: Cell cycle                      | 1.24x10^{-2} | CCND2, CDC23, SMAD2, TGFBR1, STAG2         |
| hsa04150: mTOR signaling pathway          | 1.89x10^{-2} | MAPK1, HIF1A, AKT3                         |
| hsa04620: Toll-like receptor signaling pathway | 2.02x10^{-2} | MAPK1, FOS, TOLLIP, AKT3                  |
| hsa04662: B cell receptor signaling pathway | 3.20x10^{-2} | MAPK1, FOS, AKT3                           |
| hsa04660: T cell receptor signaling pathway | 4.97x10^{-2} | MAPK1, FOS, AKT3                           |

B, IncRNA-ceRNA network

| Term                                      | P-value   | Genes                                      |
|-------------------------------------------|-----------|--------------------------------------------|
| hsa04350: TGF-beta signaling pathway      | 2.36x10^{-4} | ACVR1B, MAPK1, LTBP1, FST, TGFBR2, SMAD2, BAMB1, TGFB1 |
| hsa04068: FoxO signaling pathway          | 8.21x10^{-4} | MAPK1, SGK3, CCND2, TGFBR2, FOXO1, SMAD2, KLF2, TGFB1, AKT3 |
| hsa04668: TNF signaling pathway           | 2.07x10^{-2} | MAPK1, FOS, CEBPB, SOCS3, AKT3, ATF2       |
| hsa04931: Insulin resistance              | 2.22x10^{-2} | SOCS3, TRIB3, FOXO1, MLXIP, AKT3, NR1H3    |
| hsa05202: Transcriptional misregulation in cancer | 3.90x10^{-2} | CEBPB, CCND2, TGFBR2, FOXO1, WHSC1, PBX3, EWSR1 |

C, circRNA-ceRNA network

| Term                                      | P-value   | Genes                                      |
|-------------------------------------------|-----------|--------------------------------------------|
| hsa04350: TGF-beta signaling pathway      | 4.18x10^{-4} | MAPK1, ACVR1B, LTBP1, FST, TGFBR2, SMAD2, TGFB1 |
| hsa04520: Adherens junction               | 1.11x10^{-2} | MAPK1, ACVR1B, TGFBR2, SMAD2, SNAI1       |
| hsa04120: Ubiquitin mediated proteolysis  | 1.93x10^{-2} | SOCS3, UBE2G1, CDC23, SIAH1, HERC3, UBE2R |
| hsa04115: p53 signaling pathway           | 4.19x10^{-2} | CCND2, SIAH1, MDM4, SESN3                   |
| hsa04110: Cell cycle                      | 4.53x10^{-2} | CCND2, CDC23, SMAD2, TGFBR1, STAG2        |
| hsa05220: Chronic myeloid leukemia        | 4.53x10^{-2} | MAPK1, ACVR1B, TGFBR2, TGFB1              |
| hsa04010: MAPK signaling pathway          | 4.82x10^{-2} | MAPK1, FOS, ACVR1B, TGFBR2, MKNK2, FGF13, TGFB1 |

D, Integrated ceRNA network

| Term                                      | P-value   | Genes                                      |
|-------------------------------------------|-----------|--------------------------------------------|
| hsa04350: TGF-beta signaling pathway      | 7.61x10^{-4} | ACVR1B, LTBP1, FST, TGFBR2, SMAD2, TGFB1 |
| hsa04068: FoxO signaling pathway          | 5.92x10^{-3} | CCND2, TGFBR2, FOXO1, SMAD2, KLF2, TGFB1 |
| hsa05202: Transcriptional misregulation in cancer | 1.49x10^{-2} | CCND2, TGFBR2, FOXO1, WHSC1, PBX3, EWSR1 |
| hsa04144: Endocytosis                     | 4.72x10^{-2} | TGFBR2, CYTH4, RUFY1, SMAD2, CYTH3, TGFB1 |
| hsa04110: Cell cycle                      | 4.92x10^{-2} | CCND2, SMAD2, TGFBR1, STAG2               |

KEGG pathway analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery online tool (version 6.8; david.abcc.ncifcrf.gov) (26). ceRNAs, competing endogenous RNAs; circRNA, circular RNA; KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long noncoding RNA; miRNA, microRNA.
FOS. Furthermore, MALAT1 and hsa_circRNA_102348 could sponge hsa-miR-185-5p.

Degeneration of the intervertebral disc can be mediated by several pathways. Among them, the MAPK pathways (including JNK, ERK and p38 MAPK) have garnered extensive attention. It has been reported that blockade of p38 using Sb 202190 in NP cells can diminish the production of factors associated with inflammation, pain, and disc catabolism, and slow the course of degeneration of the intervertebral disc can be mediated by several pathways. Among them, the MAPK pathways (including JNK, ERK and p38 MAPK) have garnered extensive attention. It has been reported that blockade of p38 using Sb 202190 in NP cells can diminish the production of factors associated with inflammation, pain, and disc catabolism, and slow the course of degeneration of the intervertebral disc can be mediated by several pathways. Among them, the MAPK pathways (including JNK, ERK and p38 MAPK) have garnered extensive attention. It has been reported that blockade of p38 using Sb 202190 in NP cells can diminish the production of factors associated with inflammation, pain, and disc catabolism, and slow the course of degeneration of the intervertebral disc can be mediated by several pathways. Among them, the MAPK pathways (including JNK, ERK and p38 MAPK) have garnered extensive attention. It has been reported that blockade of p38 using Sb 202190 in NP cells can diminish the production of factors associated with inflammation, pain, and disc catabolism, and slow the course of degeneration of the intervertebral disc can be mediated by several pathways. Among them, the MAPK pathways (including JNK, ERK and p38 MAPK) have garnered extensive attention. It has been reported that blockade of p38 using Sb 202190 in NP cells can diminish the production of factors associated with inflammation, pain, and disc catabolism, and slow the course of degeneration of the intervertebral disc can be mediated by several pathways. Among them, the MAPK pathways (including JNK, ERK and p38 MAPK) have garnered extensive attention. It has been reported that blockade of p38 using Sb 202190 in NP cells can diminish the production of factors associated with inflammation, pain, and disc catabolism, and slow the course of
Furthermore, Carthamin yellow has been suggested as a promising preventative or therapeutic drug for IDD via suppression of the MAPK pathway (56), whereas Resistin mediates enhanced ECM degradation in IDD by activation of p38 MAPK (57). Accumulating evidence has demonstrated that TGFBI (34,35) and FOS (58) may exert roles via activation of the MAPK pathways in NP cells. In line with these studies, MAPK1 was highly expressed in NP samples from patients.
with IDD patients in this study. Furthermore, the present study demonstrated that MALAT1 could sponge hsa-miR-519d-3p to promote the expression of MAPK1, and hsa_circRNA_100086 could sponge hsa-miR-509-3p to upregulate MAPK1. The findings of the present study were indirectly confirmed by a previous study in osteosarcoma cells, in which MALAT1 was reported to promote osteosarcoma cell growth through the inhibition of miR-509, which leads to activation of the Rac1/JNK pathway (59).

There are some limitations to the present study. Firstly, the sample size was not large. An additional validation cohort should be included in further studies to analyze the expression of these identified lncRNAs, circRNAs, miRNAs and mRNAs. Secondly, this is a preliminary screening study and further experimental investigations are required to validate the interactions in the identified ceRNA axes in IDD.

In conclusion, the present study identified several lncRNA/circRNA-miRNA-mRNA interaction axes (MALAT1/hsa_circRNA_102348-hsa-miR-185-5p-TGFB1/FO5, MALAT1-hsa-miR-155-5p-HIF1A, hsa_circRNA_102399-hsa-miR-302a-3p-HIF1A, MALAT1-hsa-miR-519d-3p-MAPK1 and hsa_circRNA_100086-hsa-miR-509-3p-MAPK1), which may be crucial for treatment of IDD. Clinical, in vitro and in vivo experiments will be performed in future to validate the expression patterns and interactions identified in this study in IDD.

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Availability of data and materials

The datasets generated and/or analyzed during the present study are available in the Gene Expression Omnibus repository, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67567.

Authors’ contributions

JWZ, XLZ and DJH contributed to the concept and the design of the study. WJG acquired the data. JWZ, XLZ and HMH conducted the statistical analysis. XDW and DJH were involved with interpretation of the data. JWZ and XLZ drafted the manuscript. DJH revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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