Silencing NDC80, RAD21 and BUB1B ameliorates intervertebral disc degeneration by promoting proliferation and inhibiting apoptosis of nucleus pulposus cells

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Abstract

Background

Intervertebral disc degeneration (IVDD) is a commonly occurring musculoskeletal disorder, which is closely associated with low back pain. Accumulating evidence has demonstrated that dysregulated genes expression profiles play important roles in pathogenesis of IVDD. Hence, the current study was aimed to identify key genes to understand underlying mechanisms and therapeutic targets of IVDD.

Methods

Microarray datasets of GSE34095, GSE63492 and GSE45856 were downloaded to identify the hub genes that participate in the IVDD pathogenesis. After establishment of rat IVDD models, the expressions of NDC80, BUB1B and RAD21 in rat IVDD samples were evaluated by reverse transcription quantitative PCR (RT-qPCR) and immunochemistry. Subsequently, we assessed the proliferation, cycle and apoptosis of nucleus pulposus (NP) cells that transfected with siRNA-NDC80, siRNA-BUB1B and siRNA-RAD21.

Results

Our results showed indicated that NDC80, BUB1B and RAD21 were the key pathogenic genes with higher expression in IVDD rats, and silencing of NDC80, BUB1B and RAD21 gene could promote the aggrecan and collagen II synthesis, cell cycle and proliferation of NP cells, and inhibit NP cells apoptosis.

Conclusion

Our study suggests that silencing NDC80, RAD21 and BUB1B genes ameliorates intervertebral disc degeneration by promoting proliferation and inhibiting apoptosis of nucleus pulposus cells.

Background

Intervertebral disc degeneration (IVDD) is a musculoskeletal disorder characterized by imbalanced extracellular matrix synthesis and breakdown, consequently contributing to herniated disks, spine instability and spinal stenosis [1, 2]. Chronic lower back pain is considered to result from intervertebral disc degeneration, which brings a heavy economic burden to the society and families [3, 4]. At present, the medical treatment of IVDD is still limited to pain relief and delaying surgery, due to lacking of understanding of underlying pathogenesis [5, 6]. Therefore, in order to explore novel therapeutic approaches, there is an urgent need to identify the underlying molecular mechanisms of IVDD.

Except using classical experimental approaches to define IVDD, genetic factors have been considered to play emerging roles in the multifactorial etiology of IVDD [7, 8]. Recently, the dysregulated genes

expression profiles of IVDD have attracted increasing attention of researchers. Among them, MAP2K6 and RHOBTB2 are identified as two potential therapeutic targets of IVDD [9]. Periostin has also been proven to take part in the progression of human IVDD [10]. Hence, using high-throughput technology to screen out IVDD biomarkers in human samples has been shown to be promising to identify more precise criteria of disease diagnostic, classification and prognosis [11–13].

In this study, we identified NDC80, BUB1B and RAD21 genes as hub genes in the pathogenesis of IVDD utilizing the bioinformatics method. Besides, increased expression of NDC80, BUB1B and RAD21 in IVDD rats verified these genes associations with IVDD. Furthermore, we found that NDC80, BUB1B and RAD21 genes silencing could elevate the expression of aggrecan and collagen II, promote cell cycle and proliferation, and inhibit apoptosis of NP cells, which provides insight into the underlying mechanisms and therapeutic targets of IVDD.

Methods

Ethics statement

This study was performed with the approval of the Ethics Committee of Tianjin Medical University General Hospital. All animal experiments were performed under strict adherence with the Guide for the Care and Use of Laboratory Animal by International Committees.

Microarray Data Analysis

Gene expression data sets GSE34095, GSE63492 and GSE45856 were downloaded from Gene Expression Omnibus. For human NP cells in GSE34095, three degenerative samples were collected from IVDD patients and 3 non-degenerative samples were considered to be control group. MicroRNA expression profiling of GSE63492 was obtained from 5 IVDD patients, compared with 5 cadaveric discs. In relation to the miRNAs in GSE45856, three IVDD specimens and 3 traumatic intervertebral disc specimens were individually analyzed using microarray techniques. After data conversation and normalization[14], we performed differential analysis between degeneration samples and control [15]. We set $|\text{Log (fold change)}| > 1$ and adjust P value < 0.05 as the thresholds to screen out DEGs and differentially expressed miRNAs. Then, the target genes of the differentially expressed miRNAs were predicted by combining two online miRNA databases, miTarBase [16] and targetscan (<http://www.targetscan.org>).

Functional enrichment analysis of DEGs and target genes

In order to identify the underlying biological functions in IVDD, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed on the DEGs using Database for Annotation, Visualization, and Integration Discovery (DAVID) database (<https://david.ncifcrf.gov/>) [17].

Protein-protein interaction (PPI) network construction

The Search Tool for Retrieval of Interacting Genes (STRING) online tool was utilized to construct the PPI of DEGs. A confidence score > 0.4 was representative of statistical significance. Then, the visualization of PPI network was performed by Cytoscape software.

Animal grouping and modeling

A total of 25 male Sprague-Dawley (SD) rats (3 months old, 180 ± 20 g) were from the medical science experimentation center, SUN YAT-SEN UNIVERSITY (Guangzhou, China). The rats were raised individually and freely to eat and drink under 25°C conditions with humidity of 50~70% for one month. Next, 15 rats were randomly included in the IVDD group, while the remaining 10 rats were included in the normal group. The rats were anaesthetized by intraperitoneal injection with 3% pentobarbital sodium (40 mg/kg, P3761, Sigma-Aldrich Chemical Company, St Louis MO, USA). A longitudinal incision was made along the mid line of the rats back. After incision of subcutaneous tissues, the fascia surrounding the intervertebral disc was isolated and the sacrospinalis was then stripped. The spinous process (L1-6) was excised with the interlaminar ligaments cut in order to establish the IVDD rat model. Rats of the normal group were only subject to an incision of the subcutaneous tissue and suture after their operation followed by a return to normal feeding. The rats were injected with 80, 000 U/d penicillin over a period of three days. After 3 months, the rats were euthanized through an intraperitoneal injection of excessive anesthesia for follow-up experiments.

Assessment of IVDD models

Three months after the operation, microcomputed tomography (micro-CT) and magnetic resonance imaging (MRI) were used to assess the alterations to disc height and vertebral endplate with degeneration. Three rats chosen randomly from both IVDD group and normal group were euthanized through an intraperitoneal injection of excessive anesthesia. Subsequently, micro CT and sagittal T2-weighted images were obtained using SkyScan 1172 (SkyScan, Belgium) and 3.0-T MRI scanner (GE, Chicago, USA), respectively. Then, their respective intervertebral discs (L1-6) and adjacent vertebrae were collected. Histological staining including hematoxylin-eosin (HE) and safranin-O/Fast-greening staining was performed for assessment of cell morphology, extent of endplate ossification, and cartilage matrix of the endplate.

Immunohistochemistry

Three rats were randomly selected from both the IVDD and normal groups. After euthanasia, rat intervertebral disc samples were fixed with 10% formaldehyde and later cut in a successive manner into 4 μ m slice sections. The sections were permitted to react with diluted RAD21 (1: 500, ab154769, Abcam, Inc, MA, USA), RAD21 (1: 100, ab109496, Abcam, Inc, MA, USA), and BUB1B (1: 200, ab54894, Abcam, Inc, MA, USA). As for Collagen II, the climbing flake of NP cells treated for 7 d were taken, added with diluted Collagen II (1: 500, ab34712, Abcam, Inc, MA, USA). And then uniformly, the sections were added with biotin-labeled goat anti-mice secondary antibody (A21020, Abcam, USA), horseradish peroxidase-labeled

streptavidin working solution (DF7852, BIOSAMITE, Shanghai, China) and diaminobenzidine (DAB) to be observed under a light microscope.

Real-Time quantitative Polymerase Chain Reaction

Three rats were randomly selected from both the IVDD and normal groups. Then, total RNA was extracted using the trizol method. RNA was reversely transcribed into cDNA using the Takara reverse transcription kit (RR047, Takara Biotechnology Ltd., Dalian, China). RT-qPCR was performed with instructions of SYBR®Premix Ex Taq™ II kit (Takara Biotechnology Ltd., Dalian, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference.

The primer sequences were listed as follows: RAD21: CAGAGTAGAAGAGATAACCATGAG, ATTCCAAAGTCACCGAAGTC; NDC80: TGCTATGAGAAGTTCATGACTG, ATACAAGTCCTTCAGCTTCG; BUB1B: CCATGATAAAGGCATCTTCTCAG, AGTCCTCATTGAGACGCTC; Aggrecan: GCTACGGAGACAAGGATGAGTTC, CGTAAAAGACCTCACCTCCAT. GAPDH: AACTCCCATTCTTCCACC, TTGTCATACCAGGAAATGAGC; The 2- Δ Ct methods was used to calculate RNA relative quantitative results.

Isolation and cultivation of NP cells

The NP cells were collected from the remaining IVDD rats intervertebral discs (L1-6) that was digested with 0.25% trypsin (25200-056, Gibco Company, Grand Island, NY, USA) for 15 min at 37°C. The collected cells were then seeded into a 25 mm² culture flask with a density of 2×10^4 cells/mL and cultured in a 5% CO₂ incubator added with dulbecco's minimum essential medium (DMEM) medium (C11330500BT, Invitrogen, Car, Cal, USA) containing 3% fetal bovine serum (FBS) at 37°C. The third generation of NP cells were used for the following experiments.

Cell grouping and transfection of Small Interfering RNA (siRNA)

Based on the total mRNA sequences of NDC80, BUB1B and RAD21 genes (NM_006101, NM_001211 and NM_006265) in Genbank, the RNAi oligonucleotide sequences of NDC80, BUB1B and RAD21 genes (three pairs of sequences per siRNA) and the negative control (NC) sequences were designed and synthesized by Guangzhou RiboBio Co., Ltd. (Guangdong, China). The NP cells were subsequently assigned into the blank group (no transfection), NC group (transfected with empty vector), siRNA-NDC80 group (transfected with siRNA-NDC80-1/2/3), siRNA-BUB1B group (transfected with siRNA-BUB1B-1/2/3) and siRNA-RAD21 group (transfected with siRNA-RAD21-1/2/3). The siRNA with the highest transfection efficiency was selected from the 3 pairs of siRNA-NDC80, siRNA-BUB1B and siRNA-RAD21. The primer sequences for siRNA-NDC80, siRNA-BUB1B, siRNA-RAD21 are listed as follows: siRNA-NDC80-1: GCATATGAACTAGCTACTT; siRNA-NDC80-2: GCTCTCTCCTTCTGCCTTA;

siRNA-NDC80-3: GCATCTACTCAGCCTCTAA; siRNA-BUB1B-1: GCAATGAGCCTTTGGATAT; siRNA-BUB1B-2: GCTGAAGAGTACGAAGCTA; siRNA-BUB1B-3: CCAGTGTGCTTCCCAACTT; siRNA-RAD21-1:

GCCCATGTGTTTGAGTGTA; siRNA-RAD21-2: CCGCATCTATCACAGGAAA; siRNA-RAD21-3: CGGCTTATAATGCCATTA; siRNA-NC: UUCUCCGAACGUGUCACGA.

The NP cells were then implanted into a 6-well plate at a density of 2×10^5 cells/well with each well added with 1.3 mL serum-free Opti-MEM (31985-070, Gibco Company, Grand Island, NY, USA). Next, 5 μ L lipofectamine 2000 (Invitrogen Inc., Carlsbad, CA, USA) with 3 μ g of siRNA expressing plasmid were dissolved with 100 μ L serum free Opti-MEM culture medium respectively, mixed and permitted to stand at 37°C for 5 min. After that, products were added into the corresponding wells with each well containing 200 μ L corresponding products. Then, the products were placed in incubator for 5 h, and exchanged with a fresh complete medium. Finally, the cells were collected after 48-h of transfection.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cells at the logarithmic growth phase and in a well-grown state were seeded into a 96-well culture plate at a volume of 100 μ L per well with 1×10^4 cells per well. After cell adherence for 24 h, they were placed into a 5% CO₂ incubator at 37°C for further 24 h, 48 h and 72 h incubation. Later, 20 μ L MTT solution (5 mg/mL) was added into each well for incubation purposes at 37°C for 4 h. After removal of the supernatant, each well was added with 100 μ L dimethylsulfoxide (DMSO) and oscillated for 10 min. The OD value of each well at 490 nm was used to drawn the growth curve.

Flow cytometry

After 48h of transfection, the culture medium was discarded and the cells concentration was adjusted to 1×10^6 cells/mL. The cells stained with propidium iodide (PI) containing RNase (GR1-25, SBS Genetech, Beijing, China) were detected at 488 nm using a flow cytometer (FACS Calibur, Becton, Dickinson and Company, New Jersey, USA) to cell cycle. The transfected cells at the logarithmic growth phase were used to detect cell apoptosis. According to the instructions of Annexin-V-FITC/PI apoptosis kit (KA3805, Abnova, Walnut, CA, USA), flow cytometry at 488 nm using the 515 nm and 560 nm band pass filter to examine the FITC fluorescence and PI fluorescence, respectively.

Statistical analysis

One-way analysis of variance (ANOVA) was used for comparisons among multiple groups with SPSS 21.0 software. Data were expressed as mean $\pm$ standard deviation, and $p < 0.05$ was regarded as statistically significant.

Results

RAD21, NDC80 and BUB1B were identified as DEGs

Initially, bioinformatic analysis was applied to identify the differentially expressed genes (DEGs) of IVDD. In regard to GSE34095, a total of 153 DEGs were screened out, including 111 up-regulated genes and 42

down-regulated genes. Among them, the RAD21, NDC80 and BUB1B genes were upregulated (Fig. 1a). In addition, seven up-regulated miRNAs and 8 down-regulated miRNAs were obtained from GSE63492, among which miR-5100 was one of the down-regulated miRNAs (Fig. 1b). Besides, six up-regulated miRNAs and 9 down-regulated miRNAs were obtained from GSE45856. Among them, miR-1246 and miR-3908 were detected as down-regulated miRNAs (Fig. 1c). Based on the prediction analysis, the targeted genes of miR-5100, miR-1246 and miR-3908 were confirmed to be RAD21, NDC80 and BUB1B respectively. Therefore, RAD21, NDC80 and BUB1B were significantly upregulated in degenerative NP cell samples.

RAD21, BUB1B and NDC80 were the hub genes related with cell cycle

To further elucidate the molecular mechanisms of IVDD, we constructed a PPI network of DEGs from NP cells. Furthermore, it was noted that the NDC80, BUB1B, and RAD21 were the hub genes of NP cells within the PPI network (Fig. 1d).

GO enrichment analysis revealed that the RAD21 and BUB1B genes participate in the cell cycle, mitotic cell cycle, cell apoptosis, programmed cell death and cell death processes. The NDC80 gene was determined to be related to cell cycle as well as in the mitotic cell cycle (Table 1). KEGG pathway enrichment analysis revealed that the RAD21 and BUB1B genes were involved in cell cycle-related signaling (Table 2).

Table 1
The top 5 biological processes of DEGs from the PPI network

ID	Description	Pvalue	Genes
0007049	Cell cycle	0.000814171	TXNIP,CKAP2,PSMC6, RAD21 , BUB1B ,ID4, NDC80 ,NUP43,MCM6
0000278	Mitotic cell cycle	0.002794736	PSMC6, RAD21 , BUB1B , ID4, NDC80 , NUP43
0006915	Apoptosis	0.004716498	ITGB3BP,CKAP2, RAD21 ,RHOT1, BUB1B ,GJA1,PPP1R13L
0012501	Programmed cell death	0.005069056	ITGB3BP,CKAP2, RAD21 ,RHOT1, BUB1B ,GJA1,PPP1R13L
0008219	Cell death	0.010973939	ITGB3BP, CKAP2, RAD21 , RHOT1, BUB1B , GJA1, PPP1R13L
Note; Differentially expressed genes (DEGs); PPI, protein-protein interaction			

Table 2
The top 5 KEGG pathway of DEGs from the PPI network

ID	Pathway	P value	Genes
hsa04110	Cell cycle	0.004096934	RAD21, BUB1B , CDKN1B, MCM6
hsa04974	Protein digestion and absorption	0.011756318	COL9A2, COL9A3, COL3A1
hsa03030	DNA replication	0.015820249	MCM6, RFC4
hsa05162	Measles	0.032397308	FYN, TBK1, CDKN1B
hsa04151	PI3K-Akt signaling pathway	0.032726603	PDGFC,FGFR3,PPP2R3C,CDKN1B
Note: Differentially expressed genes (DEGs);KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction			

Verification Of Ivdd Rats

To assess whether a successful IVDD rat model was established, the radiologic imaging and histological staining were analyzed. Compared to the normal, the IVDD group of micro-CT scans showed decrease in disc height, increase in osteophyte formation at the vertebral edge. Besides, T2-weighted images showed significant reduction in signal intensity (Fig. 2b). As illustrated in Fig. 2c, IVDD group exhibited a significant intervertebral disc degeneration including a shrunken NP, decreased number and uneven distribution of NP cells, unclear boundary of NP and annulus fibrosis, swelling and fractured inner annulus fibrosis, irregular proliferation and calcification of the cartilage endplate. These findings proved the successful IVDD rat models.

Increased expressions of NDC80, BUB1B and RAD21 in IVDD rats

RT-qPCR and immunohistochemistry analysis were performed to examine the expression of NDC80, BUB1B and RAD21 in the intervertebral disc tissues of rats (Fig. 3). The results demonstrated that IVDD rats exhibited an elevated mRNA and protein expression of NDC80, BUB1B and RAD21, which was in agreement with the bioinformatics results.

Silencing of NDC80, RAD21 and BUB1B increased the expression of collagen II and aggrecan

After transfection for 48 h, compared to no matter the blank or NC groups, the siRNA-NDC80-1, siRNA-BUB1B-2 and siRNA-RAD21-2 groups exhibited the most distinctively down-regulated mRNA expression of NDC80, BUB1B and RAD21, which were used for observing collagen II content (Fig. 4a-c). More importantly, after transfection, the NP cells exhibited a significantly increased content of collagen II aggrecan in the siRNA-NDC80, siRNA-BUB1B and siRNA-RAD21 groups (Fig. 4d-f). These results confirmed the silence efficiency, and showed that silencing of NDC80, BUB1B and RAD21 genes could increase the synthesis of collagen II and aggrecan.

Silencing NDC80, BUB1B and RAD21 promoted proliferation of NP cells

As shown in Fig. 4G, compared with the blank group and the NC group, the proliferation rate of the siRNA-NDC80 group, the siRNA-BUB1B group and the siRNA-RAD21 group was markedly increased at the 48 h and 72 h time points (all $p < 0.05$). The above results demonstrated that silencing of NDC80, BUB1B and RAD21 genes could promote the proliferation of NP cells.

Silencing NDC80, BUB1B and RAD21 accelerated cell cycle and inhibited cell apoptosis

In terms of the cell cycle distribution and apoptosis rate between the blank and NC groups, no significant difference was found ($p > 0.05$). However, the G1/G0 phase in the siRNA-NDC80, siRNA-BUB1B and siRNA-RAD21 groups were decreased, while the S phase in these gene silencing groups were increased ($p < 0.05$). There were no obvious changes in the G2/M phase ($p > 0.05$). Furthermore, after the NDC80, BUB1B and RAD21 gene silencing, the cell apoptosis rate decreased significantly. Collectively, these results suggested that silencing of NDC80, BUB1B and RAD21 genes accelerated cell cycle, which may result in the inhibition of cell apoptosis (Fig. 5).

Discussion

Although much effort and resources have been invested, the underlying pathogenesis of IVDD still remains poorly understood. Recently, the dysregulated genes expression profiles of IVDD have attracted increasing attention of researchers. To investigate key pathogenic mechanism of IVDD, we identified NDC80, BUB1B and RAD21 as hub genes in the degenerative NP cells utilizing the bioinformatics analysis. Subsequently, increased expressions of NDC80, BUB1B and RAD21 were confirmed in IVDD rats, and silencing NDC80, BUB1B and RAD21 played a protective role in intervertebral disc degeneration by promoting proliferation and inhibiting apoptosis of nucleus pulposus cells.

According to GO enrichment analysis and KEGG analysis, NDC80, BUB1B and RAD21 were predicted to participate in several biological processes and pathways including cell death, apoptosis and cell cycle. The protein encoded by NDC80 confines itself to kinetochore and subsequently acts to mediate the formation of the kinetochore-microtubule structure, which is crucial for the stable kinetochore-microtubule interaction [18–20]. Recent studies have provided evidence highlighting a correlation between the overexpression of NDC80 and the over-activation of the mitotic checkpoint [21]. Mitotic checkpoints could prevent separation errors by adjusting the anaphase time until all of chromosomes have been precisely been attached to the spindle microtubules [22]. In mammal cells, BUB1B gene encodes BubR1 protein, which is also a vital component of mitotic checkpoints that ensures the synchrony of chromosome segregation [23]. Yamamoto et al. found that the up-regulation of BUB1B may be a physiologically compensatory mechanism to the absence of normal checkpoints function [24]. Besides, exogenous BUB1B prevents chromosomal instability in a manner of triggering apoptosis [25]. Moreover, RAD21 is one of the four subunits of cohesion, participating in repairing of DNA double-strand breaks, and growth of mitosis [26]. C-terminal cleavage of RAD21 could form degradation products, translocation of which to the cytoplasm initiates apoptosis and causes amplification of the cell death signal [27–29]. Hence,

considering the importance of three interrelated genes in chromosomal stability and increased expressions of NDC80, BUB1B and RAD21 in IVDD rats, the dysregulation of NDC80, BUB1B, and RAD21 may contribute to the disruption of mitosis and subsequent apoptosis of NP cells as IVDD progresses.

Furthermore, in vitro transfection results verified that NDC80, BUB1B and RAD21 gene silencing accelerated cell cycle progression and suppressed NP cells apoptosis. Consistent with our findings, previous studies have suggested that inhibiting of BUB1, NDC80 and RAD21 expression could regulate the cell cycle progression and apoptosis [30–33]. Additionally, the loss of extracellular matrix (ECM) such as collagen II and aggrecan has been highlighted as a marker of IVDD [34, 35]. We found that silencing NDC80, BUB1B and RAD21 could enhance synthesis of ECM component, ameliorating disc degeneration in a rat model of IVDD.

There are also some limitations in our study, which should be taken into consideration when interpreting the results. First, the underlying mechanisms on how NDC80, BUB1B and RAD21 genes silencing promoted proliferation and inhibited apoptosis of nucleus pulposus cells were not thoroughly identified. Secondly, whether there are interactions between NDC80, BUB1B and RAD21 genes or how they interact with each other are not clear. In the end, the role of NDC80, BUB1B and RAD21 genes in human intervertebral disc degeneration also needs more research.

Conclusion

In conclusion, our study identifies three hub genes of NDC80, BUB1B and RAD21 via bioinformatics analysis, and indicates that silencing of NDC80, RAD21 and BUB1B genes ameliorates intervertebral disc degeneration by promoting proliferation and inhibiting apoptosis of nucleus pulposus cells. These findings suggest that NDC80, BUB1B and RAD21 may serve as potential therapeutic targets for IVDD.

Abbreviations

IVDD: Intervertebral disc degeneration; DEGs: differentially expressed genes; NP: nucleus pulposus; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DAVID: Database for Annotation, Visualization, and Integration Discovery; STRING: Search Tool for Retrieval of Interacting Genes; micro-CT: microcomputed tomography; MRI: magnetic resonance imaging; HE: hematoxylin-eosin; siRNA: small interfering RNA; NC: negative control; RT-qPCR: Reverse transcription quantitative polymerase chain reaction.

Declarations

Ethics approval and consent to participate

This study was performed with the approval of the Ethics Committee of Tianjin Medical University General Hospital. All animal experiments were performed under strict adherence with the Guide for the Care and Use of Laboratory Animal by International Committees.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

BZ, YYG, WG and WXL: experimental work, and manuscript writing. PP, CX and JYH: data analysis, experimental work. CS and RWD: manuscript editing. XHK and SQF: research design. All authors read and approved the final manuscript.

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Figures

Figure 2

Verification of rat IVDD model by radiologic imaging and histological staining. a, Flow diagram of the experiments in vivo. b, Micro-CT (sagittal and coronal) and MRI (sagittal) were obtained 3 months after surgery. Arrows show the osteosclerosis of vertebrae (bottom left) and significant reduction in signal intensity (bottom right). c, hematoxylin-eosin and safranin-O/fast green staining

Figure 3

Expression of RAD21, NDC80 and BUB1B were upregulated in rat IVDD model. a, MRNA expression of RAD21, NDC80 and BUB1B examined by RT-qPCR. b-c, Immunohistochemistry analysis of protein expression of RAD21, NDC80 and BUB1B. * $p < 0.05$ compared with the normal group

Figure 4

The effect of silencing NDC80, BUB1B and RAD21 on the expression of collagen II and aggrecan and proliferation of NP cells. a-c, Silencing efficiency of three pairs of siRNAs in the siRNA-RAD21, siRNA-NDC80, and siRNA-BUB1B group. d-e, Silencing NDC80, BUB1B and RAD21 increased the expression of collagen II of NP cells, determined by immunohistochemistry (n = 6). f, Silencing NDC80, BUB1B and RAD21 increased aggrecan content of NP cells, determined by RT-qPCR (n = 6). g, Silencing NDC80,

BUB1B and RAD21 enhanced proliferation of NP cells, determined by MTT assay. *p < 0.05 compared with the blank and NC groups. NC, negative control; NP, nucleus pulposus

Figure 5

Silencing NDC80, BUB1B and RAD21 accelerated cell cycle and inhibited cell apoptosis of NP cells. a, cell cycle distribution in the blank, NC, siRNA-NDC80, siRNA-BUB1B and siRNA-RAD21 groups, detected by flow cytometry. b, cell apoptosis in the blank, NC, siRNA-NDC80, siRNA-BUB1B and siRNA-RAD21 groups,

detected by flow cytometry. *p < 0.05 compared with the blank and NC groups. NC, negative control; NP, nucleus pulposus

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