European Review for Medical and Pharmacological Sciences 2020; 24: 9807-9814

Wharton's Jelly-derived mesenchymal stem cells suppress apoptosis of nucleus pulposus cells in intervertebral disc degeneration *via* Wnt pathway

Y.-T. ZHAO[1], Y. QIN[2], J.-S. YANG[1], D.-G. HUANG[1], H.-M. HU[1], X.-D. WANG[1], S.-F. WU[3], D.-J. HAO[1]

[1]Department of Spine Surgery, Honghui Hospital, Xi'an Jiaotong University, Xi'an, China
[2]Honghui Hospital, Xi'an Jiaotong University, Xi'an, China
[3]Center for Translational Medicine, First Affiliated Hospital of Xi'an Jiaotong University School of Medicine, Xi'an, China

Abstract. – OBJECTIVE: Aberrant apoptosis of nucleus pulposus cells (NPCs) is one of the most remarkable pathological changes in intervertebral disc degeneration (IDD) development. Albeit the advances in the application of stem cell-based therapy in IDD treatment, the molecular mechanisms underlying the anti-apoptotic actions of mesenchymal stem cell (MSC) remain poorly elucidated.

PATIENTS AND METHODS: The expression patterns of apoptosis-related proteins and Wnt/β-catenin-related genes in NP samples isolated from patients with mild or severe IDD were compared by performing immunoblot assay and quantitative real-time polymerase chain reaction (qRT-PCR), respectively. NPCs were in vitro treated with compression to induce apoptosis and then co-cultured with Wharton's Jelly-derived MSCs without direct interaction. After that, flow cytometry was carried out to detect the apoptosis rate of NPCs and the activity of Wnt/β-catenin pathway was determined. DKK-1 was used to inhibit Wnt signaling, in prior to evaluation of the effects of WJ-MSCs on apoptosis within the co-cultured NPCs.

RESULTS: Compared to the mild IDD group, there was a significant increase in the expression of Caspase-3 and Bax in the NP tissues from severe IDD patients, whereas Bcl-2 displayed an opposite result. In addition, the expression of Wnt 3a, Wnt 5a, Wnt 10a, GSK-3β, cyclinD1 and β-catenin was notably augmented in parallel with IDD progression. After compression treatment, the proportion of apoptotic NPCs was increased, which was then dramatically reversed by WJ-MSCs co-culture. Likewise, WJ-MSCs suppressed compression-induced Wnt-related gene expression and blocking Wnt/β-catenin pathway using DKK-1 enhanced the anti-apoptotic impacts of WJ-MSCs. In the presence of DKK-1, there was no significant difference between NPCs co-cultured with WJ-MSCs and those cells cultured alone.

CONCLUSIONS: WJ-MSCs attenuate the compression-induced apoptosis in NPCs and inhibit the activation of Wnt/β-catenin signaling. Blocking Wnt/β-catenin pathway further facilitates the actions of WJ-MSCs in anti-apoptosis, indicating that Wnt/β-catenin signaling plays a crucial role in this process and may function as a potential therapeutic target for IDD treatment.

Key Words:
Mesenchymal stromal cell, Intervertebral disc degeneration, Wnt, Nucleus pulposus cell, Apoptosis

Introduction

Lower-back pain (LBP) has caused a severe socio-economic problem and becomes a leading source for disability among people below 45 years of age in developed countries[1,2]. Intervertebral disc degeneration (IDD) has been identified as a common clinical condition that is linked to the onset of LBP, although its pathogenesis is complicated and caused by multiple risk factors[3]. Numerous therapeutic strategies ranging from pain palliation to invasive operations have been developed to mitigate the progression or deterioration of IDD due to current advances in our understanding of its pathobiology[4]. Unfortunately, these treatment options are not able to effectively restore the normal physiological functions of intervertebral disc because of the non-renewable characteristic of nucleus pulposus (NP), one of the main

Corresponding Author: Dingjun Hao, MD; e-mail: zytqy@163.com

components of intervertebral disc. Besides, spine surgical interventions, including discectomy, spinal arthroplasty and electrothermal therapy, are susceptible to infection and many other complications.

Nucleus pulposus cells (NPCs) are involved in the production of extracellular matrix (ECM) components and a decrease in the number of NPCs due to apoptosis can result in the reduced ECM synthesis[5]. Consequently, it has been recognized that excessive apoptosis of NPCs contributes predominantly to IDD pathogenesis[6,7]. Several studies[8,9] have proved that mesenchymal stem cells (MSCs) differentiate into NP-like cells with elevated expression of NP-related marker genes when co-cultured with NPCs. Vadala et al[9] also demonstrated that the MSCs could restore the molecular environments of matrix, in addition to its capacities of immunomodulation and multilineage differentiation.

Wnt/β-catenin (hereafter termed as Wnt) signaling is a conserved pathway that regulates cell apoptosis and plays a vital part in osteoblast maturity during the early stage of osteogenesis[10]. Activation of Wnt/β-catenin has been reported to inhibit NPCs proliferation and trigger cell senescence thereby promoting IDD aggravation[11]. However, whether MSCs can exert effects on the apoptosis of NPCs through Wnt signaling has not been described yet.

Wharton's Jelly-derived MSCs (WJ-MSCs) are isolated in abundance from umbilical cord, a medical waste obtained during delivery, and consequently they have been deemed as more easily accessible sources of MSCs and recently identified as a substitute of bone marrow (BM)-derived MSCs in clinical application. Compared with BM-MSC population, WJ-MSCs displayed a more favorable proliferative potential but exhibited inferior differentiation capacity toward osteocytes, hemocytes, and adipocytes[12,13]. In the present study we evaluated the effects of WJ-MSCs on cell apoptosis within NPCs after exposure to compression treatment, and the pathophysiological mechanism that may be involved was also investigated. Our results will facilitate our understanding into the actions of MSCs in IDD treatment.

Patients and Methods

The degenerative and normal nucleus pulposus samples were collected from patients with IDD and healthy peers undergoing surgery due to spine injury, respectively, from January 2016 to May 2018. The degree of IDD was assessed according to the modified Pfirrmann grading system[14] by performing pre-operative magnetic resonance imaging (MRI) scans. Patients with IDD were divided into Grade II and III group (deemed as mild IDD), as well as Grade IV and V group (regarded as severe IDD), and persons with spinal tumor, infection, or malformation were excluded from our study. Written informed consents were obtained from patients and counterparts and the experimental protocol was approved by the Ethic Committee of Honghui Hospital, Xi'an Jiaotong University.

NPCs Isolation and Culture

NP tissues were dissected into pieces of 1.0 mm^3 and digested with 0.25% protease at 37°C for 1 h, followed by 0.2 mg/mL of collagenase type II for 4 h, prior to filtration through a sterilized mesh with 70 µm pore size and centrifugation at 2500 rpm for 5 min. Cells were resuspended and maintained in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12) medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in 5% CO_2. The medium was replaced every 2-3 days and the primary culture was sub-cultured when cells grew to ~80% confluence.

WJ-MSCs Isolation and Culture

The fresh umbilical cord was obtained from in a full-term neonate, followed by being washed with pre-cold phosphate-buffered saline (PBS) to remove the blood clot. Afterwards, the Wharton's jelly was isolated from umbilical cord and cut into 1 mm^3 of small fragments, which were incubated with 0.2 mg/mL type II collagenase (0.2 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 18 h and then digested using 2.5% trypsin for 30 min. Finally, the cells were first fixed with 2 mL FBS for 2 h and then maintained in 8 ml of L-DMEM medium (Hyclone, South Logan, UT, USA) containing 1% penicillin–streptomycin, 50 µg/mL L-ascorbic acid and 2 mM glutamine at 37°C in a humidified atmosphere of 5% CO_2. The culture medium was replaced every 2 days. The cells were passaged when the confluence reached 80%-90%. WJ-MSCs from passage 3 were used for determination of cell surface antigens.

Western Blotting

Primary polyclonal antibodies against human Caspase-3, Bax and Bcl-2 were purchased from Abcam (Cambridge, MA, USA). Proteins were extracted from NP tissues or cell lysates with radioimmunoprecipitation assay (RIPA) lysis buffer supplemented with protease inhibitors and phosphorylase inhibitor (Beyotime, Shanghai, China). The protein concentration was measured using the bicinchoninic acid (BCA) assay (Pierce, Appleton, WI, USA) following the manufacturer's instructions. The total proteins were resolved by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) before being transferred to polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland), which were blocked with 5% silk milk for at least 1 h and incubated overnight at 4°C with specific primary antibodies (1:1000 diluted). After rinsing with Tris Buffered Saline and Tween-20 (TBST), the membrane was then incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:3000 diluted). Finally, the labeled proteins were visualized with enhanced chemiluminescence (ECL) substrates (Millipore, Billerica, MA, USA) before quantitative analysis using AlphaView software (ProteinSimple, San Jose, CA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Assay

Total RNA was extracted from NP tissues or cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Primers of Wnt 3a, Wnt 5a, Wnt 10a, GSK-3β, cyclinD1 and β-catenin were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and listed in Table I. QRT-PCR was carried out using SYBR Green kit in a Light Cycler 480 machine (Roche, Basel, Switzerland). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the internal reference of mRNAs. The relative mRNA levels were calculated using $2^{-\Delta\Delta Ct}$ method.

Co-Culture of WJ-MSCs and NPCs

For co-culture without direct cell-cell contact, NPCs (1×10^6 cells/well) were seeded on the bottom of transwell 6-well plates, and WJ-MSC cells were placed onto the apical compartment of a 6-well polyester track-etched cell culture insert with a membrane pore size of 0.4-μm (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Sequentially, cells were grown in DMEM/F12 medium at 37 °C in a humidified atmosphere of 5% CO_2. NPCs were cultured on common plates as a control and subjected to further analysis.

Cell Counting Kit-8 (CCK-8) Assay

NPC proliferation was assessed using the CCK-8 (Dojindo Laboratories, Gaithersburg, MD, USA) according to the manufacturer's protocols. In brief, fibroblasts (5×10^3 cells/well) were plated into 96-well plates and were incubated at 37°C for 24 h before the assay. 20 μL of CCK-8 solution (5 mg/ mL) was added to each well and the cells were incubated at 37°C for another 1 h. The absorbance of samples was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Cell Apoptosis Analysis

Cells were harvested by centrifugation at 1000 rpm for 5 min, re-suspended in 100 μL binding buffer and subjected to apoptosis rate analysis using Annexin V-FITC/PI Apoptosis Detection Kit (BD Biosciences, San Diego, CA, USA). In brief, 5 μL Annexin V-FITC and 100 μL PI were added to cell cultures, followed by incubation in the dark for 15 min at room temperature and determination through flow cytometry (BD LSR II, BD Biosciences, San Diego, CA, USA).

Statistical Analysis

Data are presented as means ± SD (standard deviation). All experiments included at least 3 replicates per group. Groups were compared

Table I. Primer sequences used in qRT-PCR analysis.

Gene	Forward (5′-3′)	Reverse (5′-3′)
GSK-3β	ATCAAGGCACATCCTTGGAC	ACGGCTACACAGTGCGATT
Wnt3a	TGTTGGGCCACAGTATTCCT	ATGAGCGTGTCACTGCAAAG
Wnt5a	FCCACATGCAGTACATCGGAG	CACTCTCGTAGGAGCCCTTG
Wnt10a	AATGCCAACACCAATTCAGG	CAACTCGGTTGTTGTGAAGC
β-catenin	CATGGGTGGAACACAGCA	CCCAGTGCACCCTTCAAC
cyclinD1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
GAPDH	GATGATTGGCATGGCTTT	CACCTTCCGTTCCAGTTT

using the two-tailed Student's *t*-test for parametric data. Statistical analyses were carried out using the Statistical Product and Service Solutions (SPSS) 19.0 software (SPSS, Inc., Chicago, IL, USA). A value of $p<0.05$ was considered statistically significant.

Results

NPC Cellular Apoptosis and Wnt/β-Catenin Activation Are Linked to IDD Severity

The identification of MSCs was illustrated in Figure 1 and MSCs differentiate into NP-like cells, with elevated expression of NP-related marker genes, when co-cultured with NPCs. To determine the pathobiological changes in patients with different severity levels of IDD, NPC apoptosis and Wnt/β-catenin pathway activation were evaluated. NP tissues isolated from patients with mild or severe IDD were directly subjected to immunoblot assay measuring the apoptosis-related proteins. As shown in Figure 2A, there was a significant increase in the expression of Caspase-3 and Bax in the NP tissues from severe IDD patients as compared with the mild IDD, whereas Bcl-2 displayed an opposite result.

Next, the expression patterns of Wnt/β-catenin-related genes were assessed in NP samples from patients in different groups. QRT- PCR results indicated that there was a notable augment in the mRNA levels of Wnt 3a, Wnt 5a, Wnt 10a, GSK-3β, cyclinD1 and β-catenin within from severe IDD patients in comparison with the mild IDD group (Figure 2B), suggestive of Wnt/β-catenin signaling activation in parallel with IDD progression.

WJ-MSCs Suppress Compression-Induced Apoptosis and Wnt/β-Catenin Activation In NPCs

To reveal the molecular mechanisms underlying the protective effects of MSCs on NPC apoptosis, cells were exposed to compressive stress *in vitro* to mimic the pathological force stimulus that induces excessive cell apoptosis and contribute to IDD onset[15]. As expected, upon compression stimulus the viability of NPCs was repressed in a ti-

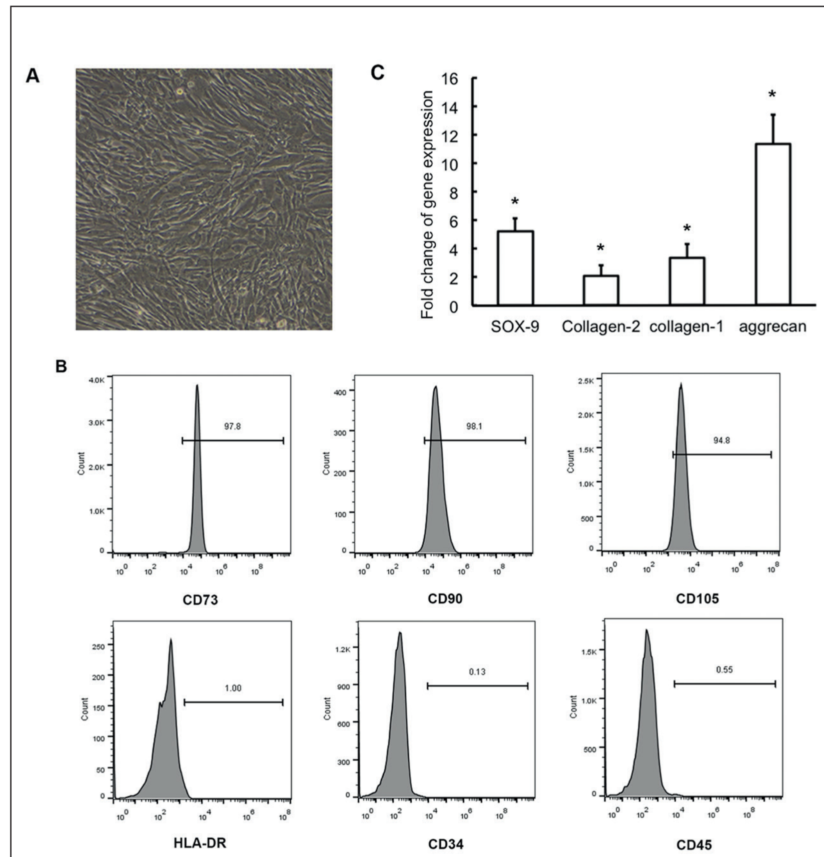

Figure 1. Identification of human WJ-MSCs. **A,** Representative image of WJ-MSC spindle-like morphology and adherence to plastic (scale bar: 50 μm, magnification: 400×) **B,** Cell surface markers (CD73, CD90, CD105, CD34, CD45 and HLA-DR) of MSC were tested by flow cytometric analysis. **C,** Relative expression levels of gene markers of NP cells were detected in co-cultured WJ-MSCs by qRT-PCR. *$p<0.05$ compared to gene expression in MSCs cultured alone.

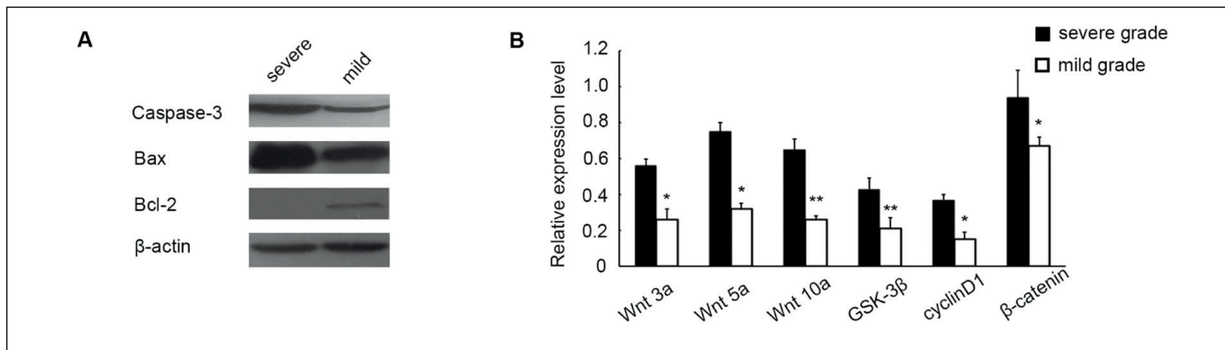

Figure 2. NPC cellular apoptosis and Wnt/β-catenin activation are linked to IDD severity. **A,** Representative Western blot results of apoptosis-related protein expression in NP samples from patients with severe or mild IDD. **B,** Expression pattern of Wnt -related genes was assessed by using qRT-PCR. Relative gene expression level was normalized by GAPDH. * indicates $p<0.05$; ** indicates $p<0.01$.

me-dependent manner and substantially retarded after 36 h- treatment (Figure 3A). The time point 36 h was therefore used in the following apoptotic examination and the proportion of apoptotic cells was significantly increased when NPCs were treated with compression (Figure 3B). Furthermore, the expression levels of above-mentioned genes involved in Wnt/β-catenin activation were evidently aggrandized after compression treatment for 36 h (Figure 3C).

As displayed in Figure 3A, for NPCs co-cultured with WJ-MSCs under compression circumstance, the number of viable cells was remarkable elevated, especially at the time point 36 h. Flow cytometry data also manifested that co-culturing with WJ-MSCs evidently mitigated the NPC

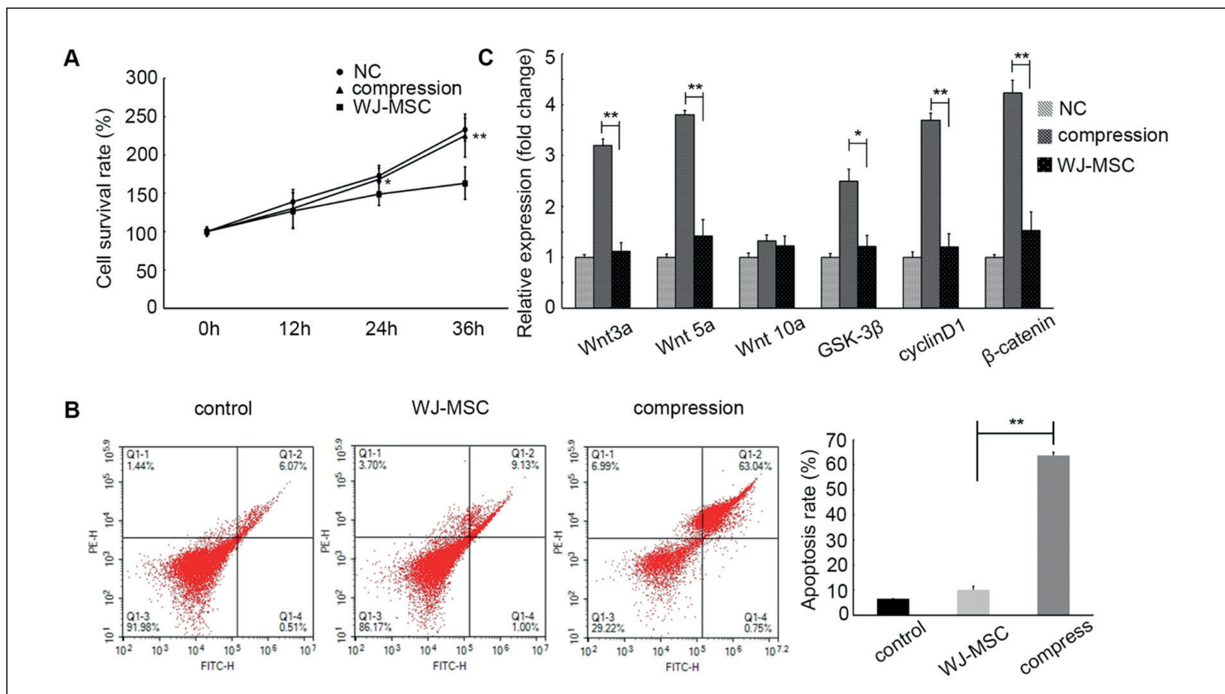

Figure 3. WJ-MSCs suppress compression- induced cell apoptosis and activation of Wnt/β-catenin in NPCs. **A,** CCK-8 was performed to determine the viability capacities of NPCs at different time intervals. The circle represents control group, triangle indicates MSC co-culture group and square means NPCs treated with compression stimulus. **B,** Fold change of Wnt-related genes in NPCs was evaluated using qRT-PCR assay after different treatments. **C,** Apoptosis rate was determined by Annexin V-FITC/PI staining. * indicates $p<0.05$; ** indicates $p<0.01$.

apoptosis compared to those cells subjected to compression stimulus alone (Figure 3B). Also, the expression of Wnt-related genes was suppressed in NPCs that co-cultured with WJ-MSCs (Figure 3C). These suggested that WJ-MSCs might exert the protective role by virtue of the activation of Wnt/β-catenin pathway.

Blocking Wnt Signaling Abrogated the Protective Actions of WJ-MSCs in Anti-Apoptosis

To verify this observation, Wnt/β-catenin signaling inhibitor DKK-1 was utilized and, as expected, we found that this led to an evident decrease in the expression of Wnt-related genes (Figure 4A). Although compression-induced apoptosis was decreased due to co-culturing with WJ-MSCs, the NPCs cultured alone exhibited a lower apoptosis rate in the presence of DKK-1 (Figure 4B). Intriguingly, once cells were pre-treated with DKK-1, there was no significant difference between NPCs cultured alone and the co-cultured cells with WJ-MSCs, indicating the role of blocking Wnt/β-catenin in protecting NPCs against apoptosis, regardless of the analogous function of WJ-MSCs. All these data suggest that Wnt/β-catenin signaling plays a predominant role in this process, and WJ-MSCs potentially suppress the compression-induced apoptosis within NPCs in a Wnt/β-catenin-dependent manner.

Discussion

Intervertebral disc is predominantly composed of annulus fibrosus and nucleus pulposus (NP), where cells are able to synthesize cartilage-specific extracellular matrix (ECM) components. Increasing evidence[16,17] manifested that excessive loss of NP cells caused by apoptosis has resulted in the interruption of ECM homeostasis, thus exacerbating the IDD progression.

Currently, MSCs have been extensively and successively applied in the treatment of IDD. MCSs are probably implicated in the degenerative disc repair in the following aspects: (I) complement damaged cells through regeneration of disc-specific cells that have the ability to produce ECM components; (II) control the inflammatory response and (III) promote tissue regeneration by virtue of paracrine signaling factors[18]. This study attempted to provide novel insight into the molecular mechanisms underlying how MSCs play their role in the therapies of IDD disease.

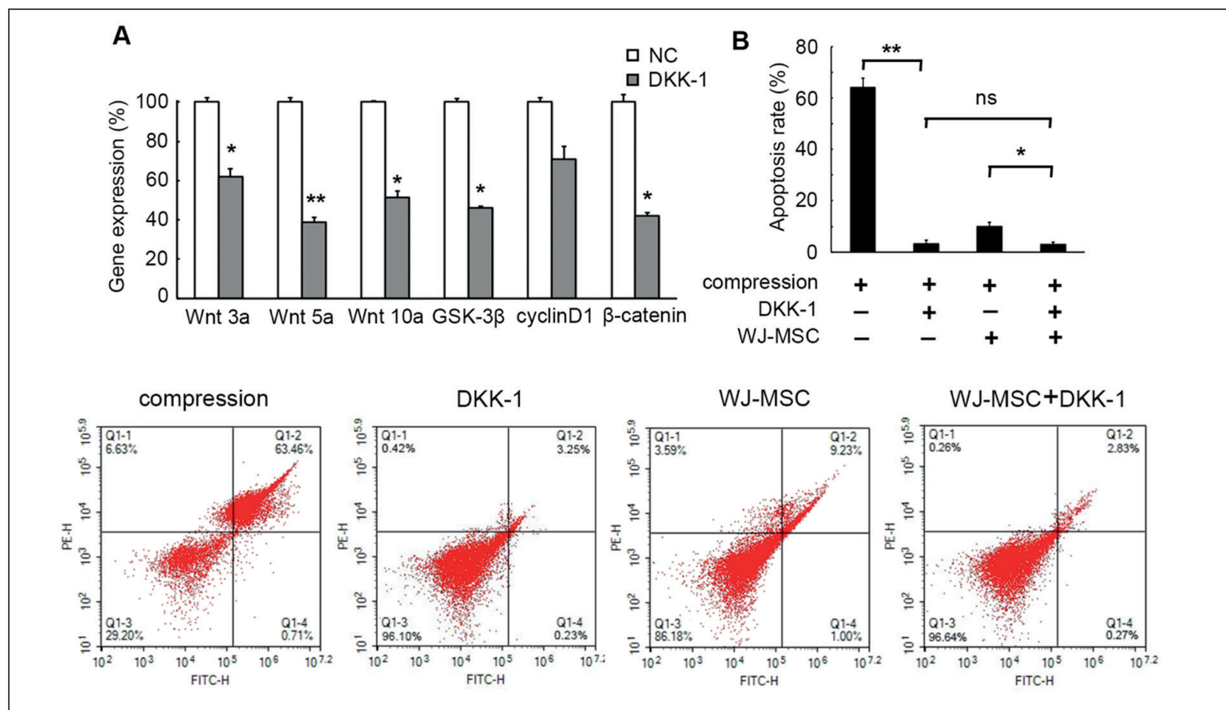

Figure 4. Inhibition of the Wnt/β-catenin signaling enhances the protective effects of WJ-MSCs. **A,** Relative expression of Wnt-related genes in NPCs was evaluated using qRT-PCR assay after DKK-1 treatment. **B,** Apoptosis rate was determined by Annexin V-FITC/PI staining after different treatments. ns represents not significant; * indicates $p<0.05$; ** indicates $p<0.01$.

The canonical Wnt/β-catenin signaling pathway is implicated in an array of cellular pathological processes including cell proliferation, apoptosis and differentiation[18]. In particular, the involvement of Wnt/β-catenin signaling in the pathogenesis of IDD has been currently revealed. However, different researches arrive at contradictory results considering the role of Wnt signaling in mediating this degenerative disease. By performing gene expression profiling, Smolders et al[19] found that *Caveolin-1* gene was significant down-regulated in the process of cellular transformation from NP notochordal cells into chondrocyte-like cells. The authors also claimed that *Caveolin-1* knockout led to excessive cell apoptosis within the NP tissue. These suggested that the down-regulated Wnt signaling was involved in the early stage of IDD development. Sun et al[20] indicated that Wnt/β-catenin was suppressed by miR-532 and suppression of Wnt/β-catenin signaling remarkably boosted miR-532-induced NPC cell apoptosis. Inversely, Wnt/β-catenin was down-regulated by aquaporin 3, a water channel protein that restrains NPC apoptosis, as well as ECM breakdown, and the protective function of aquaporin 3 was reversed due to activation of Wnt/β-catenin signaling[21]. Hiyama et al[22-24] reported that Wnt signaling activation could not only retarded NPCs proliferation and promoted cell senescence, but TNF-α expression was also up-regulated and the initiation of inflammatory response further enhanced Wnt signaling activation.

Beyond these contradictory results showing the important role of Wnt signaling pathway in IDD development, the present study indicated that Wnt/β-catenin in NPCs was enhanced upon compression stimulation, but a marked decline in the expression of Wnt-related genes was found when co-cultured with WJ-MSCs. This implies that WJ-MSCs may exert the protective effects on NPC cell apoptosis through inhibition of Wnt/β-catenin pathway. Given that MSCs and NPCs were co-cultured without cell-cell interaction, it was suggested that MSCs modulate cell apoptosis *via* paracrine signaling transduction. Afterwards, Wnt signaling inhibitor DKK-1 was utilized and it resulted in an evident decrease in the compression-induced Wnt-related genes. Intriguingly, once cells were pre-treated with DKK-1, levels of all these genes expressed in NPCs co-cultured with WJ-MSCs were analogous to those NPCs cultured alone.

Considering the apoptosis percentage, NPCs exposed to DKK-1 displayed values lower than those observed in absence of it, which indicated that inactivation of Wnt/β-catenin pathway protected NPCs against detrimental cell apoptosis. Co-culture with WJ-MSCs might have impact on other signaling pathways that interplay with Wnt/β-catenin during this process, as Han et al reported[25]. Anyway, there was barely detectable difference in apoptosis rate between NPCs co-cultured with WJ-MSCs and those cells cultured alone, once Wnt/β-catenin signaling was blocked. That is, Wnt/β-catenin signaling plays a predominant role in this process, and WJ-MSCs potentially suppress the compression-mediated apoptosis within NPCs in a Wnt/β-catenin-dependent manner.

Conclusions

Taken together, our data manifest that after co-culture with WJ-MSCs, compression-induced cell apoptosis within NPCs was significantly diminished, and the activity of Wnt/β-catenin pathway was also decreased, indicating WJ-MSCs potentially protect NPC cells from apoptosis by retarding Wnt/β-catenin activation. Blocking Wnt/β-catenin signaling markedly enhanced the performance of WJ-MSCs in anti-apoptosis. All these suggest that WJ-MSCs mitigate cell apoptosis within intervertebral disc and thus alleviate the degenerative process by predominantly inhibiting Wnt/β-catenin signaling pathway, which may serve as a potential therapeutic target for IDD treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Acknowledgements

China Postdoctoral Science Foundation (2016M602943XB); Shaanxi Postdoctoral Research Fund (2017BSHQYX-MZZ19).

References

1) Kadow T, Sowa G, Vo N, Kang JD. Molecular basis of intervertebral disc degeneration and herniations: what are the important translational questions? Clin Orthop Relat Res 2015; 473: 1903-1912.
2) Mathew J, Singh SB, Garis S, Diwan AD. Backing up the stories: the psychological and social costs of chronic low-back pain. Int J Spine Surg 2013; 7: e29-e38.

3) Luoma K, Riihimaki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A. Low back pain in relation to lumbar disc degeneration. Spine (Phila Pa 1976) 2000; 25: 487-492.
4) Zeng Y, Chen C, Liu W, Fu Q, Han Z, Li Y, Feng S, Li X, Qi C, Wu J, Wang D, Corbett C, Chan BP, Ruan D, Du Y. Injectable microcryogels reinforced alginate encapsulation of mesenchymal stromal cells for leak-proof delivery and alleviation of canine disc degeneration. Biomaterials 2015; 59: 53-65.
5) Phillips KL, Cullen K, Chiverton N, Michael AL, Cole AA, Breakwell LM, Haddock G, Bunning RA, Cross AK, Le Maitre CL. Potential roles of cytokines and chemokines in human intervertebral disc degeneration: interleukin-1 is a master regulator of catabolic processes. Osteoarthritis Cartilage 2015; 23: 1165-1177.
6) Ding F, Shao ZW, Xiong LM. Cell death in intervertebral disc degeneration. Apoptosis 2013; 18: 777-785.
7) Kepler CK, Ponnappan RK, Tannoury CA, Risbud MV, Anderson DG. The molecular basis of intervertebral disc degeneration. Spine J 2013; 13: 318-330.
8) Vadala G, Studer RK, Sowa G, Spiezia F, Iucu C, Denaro V, Gilbertson LG, Kang JD. Coculture of bone marrow mesenchymal stem cells and nucleus pulposus cells modulate gene expression profile without cell fusion. Spine (Phila Pa 1976) 2008; 33: 870-876.
9) Vadala G, Russo F, Ambrosio L, Loppini M, Denaro V. Stem cells sources for intervertebral disc regeneration. World J Stem Cells 2016; 8: 185-201.
10) Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med 2013; 19: 179-192.
11) Hiyama A, Sakai D, Risbud MV, Tanaka M, Arai F, Abe K, Mochida J. Enhancement of intervertebral disc cell senescence by WNT/beta-catenin signaling-induced matrix metalloproteinase expression. Arthritis Rheum 2010; 62: 3036-3047.
12) Batsali AK, Pontikoglou C, Koutroulakis D, Pavlaki KI, Damianaki A, Mavroudi I, Alpantaki K, Kouvidi E, Kontakis G, Papadaki HA. Differential expression of cell cycle and WNT pathway-related genes accounts for differences in the growth and differentiation potential of Wharton's jelly and bone marrow-derived mesenchymal stem cells. Stem Cell Res Ther 2017; 8: 102.
13) Wang Q, Yang Q, Wang Z, Tong H, Ma L, Zhang Y, Shan F, Meng Y, Yuan Z. Comparative analysis of human mesenchymal stem cells from fetal-bone marrow, adipose tissue, and Warton's jelly as sources of cell immunomodulatory therapy. Hum Vaccin Immunother 2016; 12: 85-96.
14) Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. Spine (Phila Pa 1976) 2001; 26: 1873-1878.
15) Ding F, Shao ZW, Yang SH, Wu Q, Gao F, Xiong LM. Role of mitochondrial pathway in compression-induced apoptosis of nucleus pulposus cells. Apoptosis 2012; 17: 579-590.
16) Park JB, Lee JK, Park SJ, Kim KW, Riew KD. Mitochondrial involvement in fas-mediated apoptosis of human lumbar disc cells. J Bone Joint Surg Am 2005; 87: 1338-1342.
17) Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol 2014; 10: 44-56.
18) Gordon MD, Nusse R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem 2006; 281: 22429-22433.
19) Smolders LA, Meij BP, Onis D, Riemers FM, Bergknut N, Wubbolts R, Grinwis GC, Houweling M, Groot KM, van Leenen D, Holstege FC, Hazewinkel HA, Creemers LB, Penning LC, Tryfonidou MA. Gene expression profiling of early intervertebral disc degeneration reveals a down-regulation of canonical Wnt signaling and caveolin-1 expression: implications for development of regenerative strategies. Arthritis Res Ther 2013; 15: R23.
20) Sun Z, Jian Y, Fu H, Li B. MiR-532 downregulation of the Wnt/beta-catenin signaling via targeting Bcl-9 and induced human intervertebral disc nucleus pulposus cells apoptosis. J Pharmacol Sci 2018; 138: 263-270.
21) Xie H, Jing Y, Xia J, Wang X, You C, Yan J. Aquaporin 3 protects against lumbar intervertebral disc degeneration via the Wnt/beta-catenin pathway. Int J Mol Med 2016; 37: 859-864.
22) Hiyama A, Sakai D, Tanaka M, Arai F, Nakajima D, Abe K, Mochida J. The relationship between the Wnt/beta-catenin and TGF-beta/BMP signals in the intervertebral disc cell. J Cell Physiol 2011; 226: 1139-1148.
23) Hiyama A, Sakai D, Risbud MV, Tanaka M, Arai F, Abe K, Mochida J. Enhancement of intervertebral disc cell senescence by WNT/beta-catenin signaling-induced matrix metalloproteinase expression. Arthritis Rheum 2010; 62: 3036-3047.
24) Hiyama A, Yokoyama K, Nukaga T, Sakai D, Mochida J. A complex interaction between Wnt signaling and TNF-alpha in nucleus pulposus cells. Arthritis Res Ther 2013; 15: R189.
25) Han Z, Wang J, Gao L, Wang Q, Wu J. Aberrantly expressed messenger RNAs and long noncoding RNAs in degenerative nucleus pulposus cells co-cultured with adipose-derived mesenchymal stem cells. Arthritis Res Ther 2018; 20: 182.