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JNK phosphorylation promotes natural degeneration of cervical endplate chondrocytes by down-regulating expression of ANK

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Abstract. – BACKGROUND: Endplate degeneration leads to accelerated degeneration of the intervertebral disc. The importance of endplate chondrocytes in this process is unclear. Many cellular processes in chondrocytes are controlled by activated c-Jun N-terminal kinases (JNK) and protein kinase B (AKT). However, the involvement of their pathways in the degeneration process needs to be elucidated.

AIM: To study activation of JNK and AKT signaling pathways and their significance for degeneration of endplate chondrocytes, as well as involvement of progressive ankylosis protein (ANK) in this process.

MATERIALS AND METHODS: Rat primary chondrocytes were grown to confluence and subcultured until passage 4. Morphological appearances (microscope, hematoxylin & eosin staining, toluidine blue staining) and proliferation rates of cells (MTT test) were observed. Further, levels of type II collagen, aggrecan, phosphorylated JNK and AKT, total JNK, AKT and ANK were evaluated by qPCR, flow cytometry and Western blot assays. Furthermore, inhibition experiments with SP600125, the JNK inhibitor, were carried out in the passage 4 cells to assess the effects of the JNK pathway on natural degeneration of endplate chondrocytes.

RESULTS: The proliferative speed of endplate chondrocytes progressively decreased during passaging. Expressions of type II collagen and aggrecan were significantly decreased with cells at higher passages. Furthermore, phosphorylation of JNK, but not AKT, was significantly upregulated and accompanied by reduced ANK expression. Inhibition of the JNK pathway increased expression of type II collagen, aggrecan and ANK and facilitated proliferation rates.

CONCLUSIONS: Phosphorylation of JNK promotes natural degeneration of cervical endplate chondrocytes, likely by down-regulating ANK expression.

Key Words:
Phosphorylation, JNK, AKT, ANK, Chondrocytes, Intervertebral disc degeneration, Proteoglycans, Collagen.

Abbreviation

JNK = c-Jun N-terminal kinases; AKT = protein kinase B; ANK = progressive ankylosis protein; IVD = intervertebral discs; ECM = extracellular matrix; MAPK = mitogen-activated protein kinase; AKT = protein kinase B; AMPK = AMP-activated protein kinase; ERK = extracellular-signal-regulated kinase; PKC = protein kinase C; TGF-β1 = Transforming Growth factor-β1; GAPDH = glyceraldheyde 3-phosphate dehydrogenase; FITC = fluorescein isothiocyanate; FBS = fetal bovine serum; MMPs = matrix metalloproteinases; iNOS = inducible nitric oxide synthase; PBS = phosphate buffered saline.

Introduction

Degeneration of intervertebral discs (IVD) in the cervical spine plays a central role in the pathogenesis of discogenic pain, disc herniation, spinal instability and stenosis, and facet joint osteoarthritis. However, the exact pathogenesis of IVD degeneration still remains unclear. Previous studies demonstrated that IVD are the largest avascular tissue in the body. The nutrient supply of disc cells mainly depends on diffusion from the cartilage endplate[1-5]. The endplate cartilage is a layer of hyaline cartilage, approximately 0.6-mm thick, lying between the vertebral body and the IVD. In the past decade, increasing number of studies showed that IVD degeneration begins in the cartilage endplate[2,6-10]. Endplate chondro-

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cytes are unique cells dispersed in extracellular matrix (ECM) of the dense cartilage endplate; these cells are abundant of type II collagen and aggrecan. Endplate chondrocytes are responsible for both synthesis and degradation of ECM. Therefore, studies of the endplate chondrocyte pathophysiology may provide novel insights into the pathogenesis of IVD degeneration.

c-Jun N-terminal kinases (JNK) are now recognized as one of the most important determinants of cellular apoptosis in chondrocytes[11-13]. JNK belong to the family of Mitogen-Activated Protein Kinases (MAPK) which mediate cellular responses to a variety of intracellular and extracellular stresses. JNK proteins are encoded by three different genes named jnk1, jnk2 and jnk3. The jnk1 and jnk2 genes are expressed in all cells, including chondrocytes[14,15]. By contrast, the jnk3 gene has a more specific pattern of expression and is largely restricted to brain, heart, and testis. The biological roles of JNK include regulation of stress responses and inflammation, and control of cell proliferation, differentiation, apoptosis and degeneration[16].

Another determinant of chondrocyte survival is protein kinase called AKT[11,17,18]. Studies from the past decade showed that AKT is involved in numerous cellular function including transcriptional regulation, cell growth, nutrition metabolism, proliferation, apoptosis, and survival ability[17-24]. There are three AKT genes identified in mammalian cells. AKT1 is located on chromosome 14q32, while AKT2 and AKT3 on chromosomes 19q13 and 1q44, respectively. AKT1 and AKT2 are expressed in all cell types, whereas AKT3 exhibits a more tissue-specific pattern of expression. The three AKT isoforms share a conserved domain structure and have 80% similarity in amino acid sequence[25]. The roles of both JNK and AKT in degeneration of endplate chondrocytes remains to be elucidated.

There have been reports that progressive ankylosis protein (ANK) controls pyrophosphate levels and, thereby, tissue calcification, also in cartilage[26]. Specifically, the ANK gene encodes a 492-amino acid long multipass transmembrane protein that regulates the intracellular inorganic pyrophosphate (PPi) steady-state concentration. ANK may function as a transporter to shuttle PPi ions across the plasma membrane to the extracellular environment[26,27]. It has been previously demonstrated that ANK is positively associated with cartilage endplate and cartilage endplate chondrocytes degeneration[28,29]. However, the relationship between JNK, AKT, and ANK in the progress of degeneration of endplate chondrocytes is still largely unknown.

In the present study, we studied this relationship using a model of natural degeneration of primary rat cervical endplate chondrocytes. We found that phosphorylation of JNK, but not AKT, promotes natural degeneration of cervical endplate chondrocytes, possibly by down-regulation of ANK expression. Thus, our study provides evidence for functional regulation of the cervical cartilage endplate and identifies new targets for prevention of IVD degeneration.

Materials and Methods

Animals

Six-week-old male Sprague-Dawley rats (160-180 g) were purchased from the Experimental Animal Centre of Chinese Academy of Science (Shanghai, CA, China). All animals were housed in the pathogen-free rat colonies. The study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Medical Laboratory Animals (Ministry of Health, P. R. China, 1998). The study protocol was approved by the Medical Laboratory Animal Care and Use Committee of Anhui Province, and the Ethics Committee of Yijishan Hospital of Wannan Medical College.

Chondrocytes Isolation and Culture

Primary rat chondrocytes were isolated and cultured as described elsewhere[29]. In brief, endplate chondrocytes were grown to confluence, trypsinized and passaged into new dishes until the cells reached passage four. The culture medium was changed every 3 days. Using primary endplate chondrocytes as control group (passage 0), other passages of cells formed four different experiment groups (i.e., passages 1 to 4). The culture conditions and seeding density of each passage cells were the same as with the primary cells at passage 0. The cell morphology was observed throughout the entire culture interval using inverted phase contrast microscope and the hematoxylin & eosin and toluidine blue stainings.

The chondrocytes Proliferation Assay

Chondrocytes were seeded in 96-cell plates at a density of 5×10^3 cells/well and cultured for 11 days in DMEM/F-12 (Sigma, St. Louis, MO,

USA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA). Culture medium was changed every 3 days. The cells were washed with phosphate-buffered saline (PBS) and incubated with medium containing 50 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, St. Louis, MO, USA) for 4 hours at 37° C. After incubation, the cells were washed again with phospate buffered saline (PBS) and solubilized using 200 μl of dimethyl sulfoxide (DMSO). The resulting intracellular purple formazan was quantified by measuring the absorbance at a wavelength of 492 nm using Model 680 Microplate Reader (Bio-Rad, Hercules, CA, USA).

Hematoxylin and Eosin Staining

The cells were rinsed three times with PBS times and fixed with 4% paraformaldehyde for 30 min at room temperature. The fixed cells were rinsed for 10 min with running tap water and for 5 min with distilled water. Then, the fixed cells were incubated with hematoxylin solution for 8 min at room temperature. The stained cells were rinsed with running tap water for 30 sec and incubated with eosin solution for 5 min at room temperature. Finally, the cells were washed with distilled water and observed under 80I Fluorescence Microscope (Nikon, Tokyo, Japan).

Toluidine Blue Staining

The first staining steps were the same as with the hematoxylin and eosin staining. Then, the fixed cells were incubated with toluidine blue solution for 4 hours at room temperature, washed with distilled water, and observed under fluorescence microscope.

Inhibition of the JNK Signaling Pathway

When the passage 4 rat endplate chondrocytes reached 80% confluence, the cultured cells were pretreated with serum-free DMEM/F-12 for 12 hours to synchronize. Then, cells were pre-incubated with or without SP600125 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), the JNK inhibitor (10 μM for 1 hour). qPCR, flow cytometry and Western blot analyses were performed to study expressions of ANK, type II collagen, and aggrecan, as well as the levels of phosphorylated (p)-JNK and total JNK.

Furthermore, the passage 4 cells were cultured with or without SP600125 for 11 days to observe the long-term morphological changes and cell proliferation rates.

qPCR Analysis

Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After reverse transcription, qPCR was performed using SYBR Premix Ex Taq kit (Takara, Dalian, USA) and Roche LightCycler 480 system (Roche, Salt Lake City, UT, USA) according to manufacturers' instructions. The qPCR conditions were as follows: denaturation at 95°C for 10 sec followed by 40 cycles of 95°C (10 sec) and 60°C (30 sec). The dissociation stage was added at the end of amplification procedure to reveal any non-specific amplification. Expression of GAPDH was used as an internal control. Data were analyzed using the $2^{-\Box\Box Ct}$ method[30] and expressed as fold change compared to respective control. Each sample was analyzed in triplicate. The primer sequences are shown in Table I.

Flow Cytometry Analysis

Chondrocytes were collected by trypsinization and washed with PBS. Cells were stained with anti-ANK FITC-conjugate monoclonal antibody (Cell Signaling Technology, Beverly, MA, USA) diluted in PBS (1% FBS). For intracellular staining, cells were fixed and permeabilized using fix-

Table I. Primer sequences for SD rat genes used in qPCR.

Genes	Forward primer	Reverse primer	Referencea	Product length (bp)
GAPDH	CTCAACTACATGGTCTACATGTTCCA	CTTCCCATTCTCAGCCTTGACT	NM_017008	81
Type II collagen	CCTGAAACTCTGCCACCCAG	GTTCTTCCGAGGCACAGTCG	NM_012929	151
Aggrecan	ACACCCCTACCCTTGCTTCT	AAAGTGTCCAAGGCATCCAC	NM_022190	124
ANK	CAAGAGAGACAGGGCCAAAG	AAGGCAGCGAGATACAGGAA	NM_053714	173

Footnote: [a]Gene bank access number.

ation buffer and permeabilization solution (eBioscience, San Diego, CA, USA) and stained with the following antibodies, all diluted in PBS (1% FBS): p-JNK and total JNK (both from Santa Cruz Biotechnology), p-AKT and total AKT (Cell Signaling Technology). Cells were analyzed using FACS (Beckman Coulter, Fullerton, CA, USA) and Cell Quest software (Becton Dickinson Bioscience, San Diego, CA, USA).

Western Blot Analysis

For Western blot analysis, 20 μg of cell lysate were resolved by SDS-PAGE on 10% polyacrylamide gel, transferred onto a nitrocellulose membrane (Whatman, Piscataway, NJ, USA), and probed sequentially with specific monoclonal antibodies against p-JNK and total JNK, p-AKT and total AKT, as well as ANK (the antibody suppliers as before). All primary antibodies were used at a dilution of 1:1000. As a gel-loading control, the levels of □-actin expression were determined using anti-actin antibody (Cell signaling Technology) at 1:2000 dilution. Membranes were then incubated with secondary goat anti-mouse or goat anti-rabbit horseradish peroxidaseconjugated antibodies (1:10,000 dilution; Jackson Immunoresearch Laboratories, Baltimore, MD, USA) and developed using ECL kit (Amersham Biosciences, Uppsala, Sweden).

Statistical Analysis

All data are presented as mean ± SD of at least three separate experiments. For statistical analysis of the differences, we utilized two-tailed independent Student's *t*-test, ANOVA analysis, or the Spearman's rank correlation test. All statistical analyses were done with help of SPSS, version 12.0 (SPSS Inc., Chicago, IL, USA). The p value of < 0.05 was considered statistically significant.

Results

Cell Proliferation Rates and Morphological Changes in Naturally Degenerating Rat Endplate Chondrocytes

The primary endplate chondrocytes in culture medium were spherical and refracted light when observed under inverted phase contrast microscope. The cells gradually adhered to the growth surface over the period of 24 hours and changed their form to polygons that possessed one or two large and round nuclei, and abundant cytoplasm with secretory granules. After being cultured for 10-13 days, the cells resembled cobblestones when viewed in the sites of high density. The subsequently passaged cells required less time to adhere, and the cells proliferated more quickly. The cells of passage 1 to 4 completely adhered within 24 hours and finished monolayer fusion within 3-5 days. These cells maintained their polygonal shape up until the passage 3. However, as the passage number increased, the cell proliferation rate was gradually down-regulated (Figure 1A). At passage 4, cells became degenerative. The majority of the cells displayed morphological changes by becoming spindle-shaped and showing a tendency to fibrosis (Figure 1B).

Phenotypic Identification

The mRNA expression levels of cartilaginous marker genes type II collagen and aggrecan were evaluated by qPCR. As shown in Figure 1C, mRNA expression levels of both type II collagen and aggrecan increased from primary cells to the passage 3 cells and significantly decreased with the passage 4 cells ($p < 0.01$ vs. primary cells), indicating that primary endplate chondrocytes gradually lose their native phenotype during prolonged *in vitro* expansion. The toluidine blue staining revealed a purple or red metachromatic glycoprotein secretion around the cell and in the cytoplasm (Figure 1Bγ).

Phosphorylation of JNK and AKT in Naturally Degenerating Rat Endplate Chondrocytes

Subsequent to above experiments, we explored the regulatory roles of JNK and AKT in the progress of natural degeneration of cervical endplate chondrocytes. We found that JNK phosphorylation levels increased during the course of passaging, with the most significant increase observed in the passage 4 cells ($p < 0.01$ vs. primary cells; Figure 2A and 2B). However, phosphorylation levels of AKT did not change during cell degeneration ($p > 0.05$ vs. primary cells; Figure 2B).

Expression of ANK in Naturally Degenerating Rat Endplate Chondrocytes

Next, we documented the kinetics of expression of ANK during natural degeneration of rat endplate chondrocytes. We found that expression of ANK, both protein and mRNA, significantly and progressively decreased until passage 4 ($p < 0.01$ vs. primary cells; Figures 3A, 3B, and 3C). Further, the correlation analysis showed that expres-

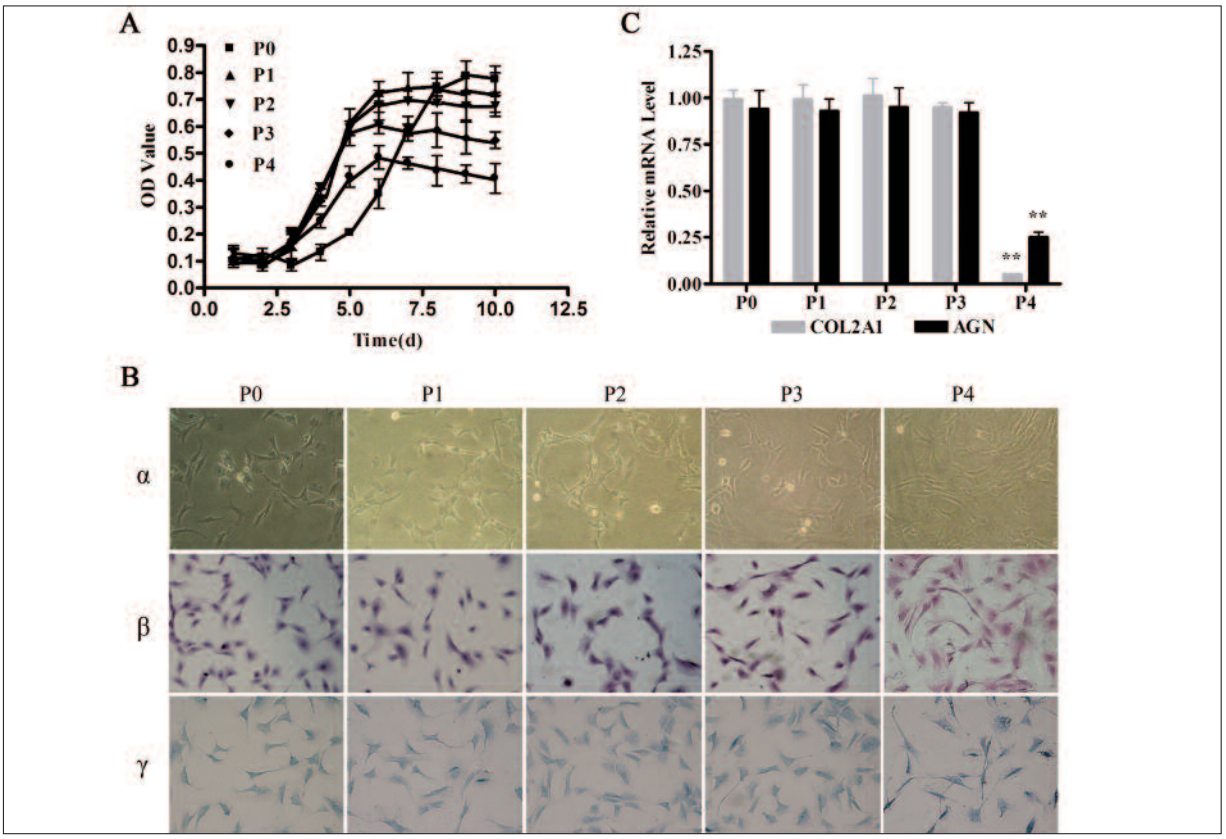

Figure 1. Proliferation curves, morphological changes and mRNA expression patterns in naturally degenerating rat endplate chondrocytes. Endplate chondrocytes were grown to confluency, trypsinized and repetitively subcultured into new dishes until the cells reached passage 4. ***A***, Endplate chondrocytes from P0 to P4 were analyzed for 11 days after each subculture; absorbances of serially passaged cells were plotted. ***B,*** Morphology of endplate chondrocytes was observed using inverted phase contrast microscope (α, magnification × 200), when hematoxylin & eosin stained (β, magnification × 200), or toluidine blue stained (γ, magnification × 200). The toluidine blue staining reveals purple or red metachromatic glycoprotein secretion around the cell and in the cytoplasm of endplate chondrocytes. Cells in each passage were cultured for 3 days, with the exception of primary cells that were cultured for 7 days. ***C,*** mRNA expression levels in the P0-P4 rat endplate chondrocytes were quantified by qPCR. Results were obtained in 3 independent experiments, and representative results are shown in panel B. Data are shown as mean ± SD. $^{**}p < 0.01$ vs. primary cells. P0: primary cells; P1-P4: passage 1-4 cells.

sion of p-JNK negatively correlated with the expression of ANK in the progress of natural degeneration of rat endplate chondrocytes ($r = –0.967$, $p < 0.01$; Figure 3D).

Effects of Inhibition of the JNK Pathway by SP600125 on Degeneration of Endplate Chondrocytes

As demonstrated above, phosphorylation levels of JNK increase, while expressions of ANK, type II collagen and aggrecan decrease during sequential cell passaging, indicating that phosphorylation of JNK, but not AKT, is a crucial event for natural degeneration of cervical endplate chondrocytes. Based on these observations, we next hypothesized that the JNK pathway plays a role in the progress of natural degeneration of endplate chondrocytes by regulating expression of ANK. This was tested in experiments which utilized SP600125, the inhibitor of JNK pathway.

In the passage 4 cells, we observed that SP600125 significantly reduced phosphorylation levels of JNK and increased expressions of ANK, type II collagen and aggrecan ($p < 0.01$ for p-JNK, < 0.05 for ANK, type II collagen and aggrecan; all comparisons vs. primary chondrocytes; Figures 4A, 4B, and 4D). Furthermore, we found that SP600125 was able to reverse proliferation rates of endplate chondrocytes observed during their natural degeneration (Figure 4C). These observed findings implicated the JNK pathway in the progress of natural degeneration of cervical endplate chondrocytes, possibly through regulating ANK gene.

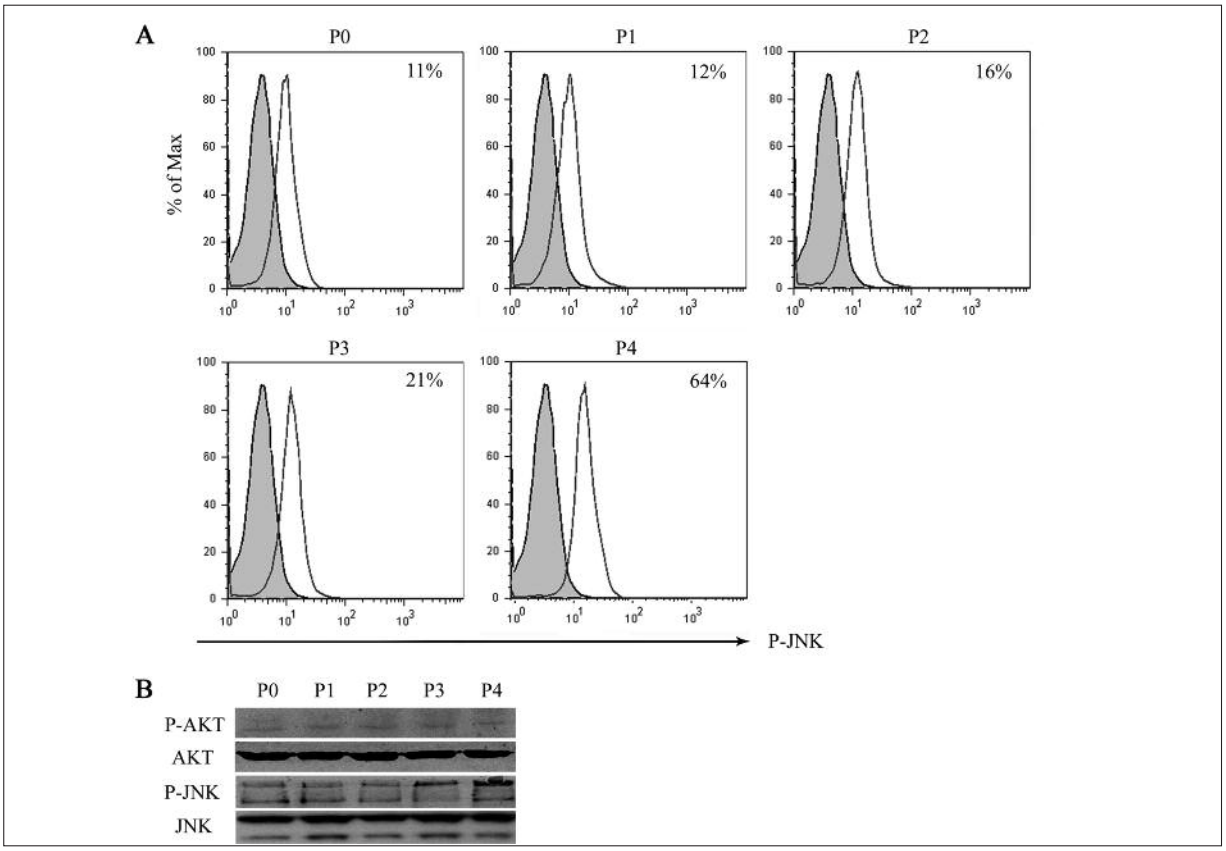

Figure 2. Phosphorylation of JNK (p-JNK) and AKT (p-AKT) in naturally degenerating rat endplate chondrocytes. Endplate chondrocytes were grown to confluency, trypsinized and repetitively subcultured into new dishes until the cells reached P4. ***A,*** Kinetics of expression levels of p-JNK and p-AKT in the P0-P4 endplate chondrocytes were analyzed by flow cytometry. ***B,*** Kinetics of expression levels of p-JNK and p-AKT in the P0-P4 endplate chondrocytes were analyzed by Western blot. Results were obtained in 3 independent experiments, and representative results are shown. P0: primary cells; P1-P4: passage 1-4 cells.

Discussion

The IVD degeneration is thought to underlie the neck and back pain. The cartilaginous endplate, a critical component in the transport pathway, shows early signs of degeneration with cracks and irregularities, which may be responsible for degeneration of the disc[2,6-10]. Chondrocytes in the cartilage endplate are unique cells, which are responsible for both synthesis and degradation of the cartilage endplate extracellular matrix. The responses by endplate chondrocytes define IVD degeneration.

Studies demonstrate that extended serial expansion of chondrocytes leads to a rapid loss of their original phenotype; these aged cells are characterized by decreased capacity for biosynthesis and deposition of the endplate cartilage-specific extracellular matrix, such as type II collagen and aggrecan. Further, these cells lose their original round or polygonal appearance and become spindle-shaped[31-33]. In line with previous reports, we also found that endplate chondrocytes became fusiform and their proliferative speed decreased during the course of serial passaging. The expression of type II collagen and aggrecan also significantly decreases when the cells are passaged to passage 4. Alini et al[34] stated that animal models may be useful in studying some specific aspect of disc biology. Our results indicate that the serially *in vitro* expanded rat endplate chondrocytes also can serve as a useful model to study the progress of natural degeneration of endplate chondrocytes.

c-Jun N-terminal kinases (JNK) are members of MAPK. There are strong evidences that phosphorylated JNK involves the progress of chondrocytes apoptosis[11-13]. For example, chondrocytes can be protected from apoptosis by inhibiting the JNK pathway[12]. However, it was not clear whether phosphorylated JNK is involved in degeneration of cervical endplate chondrocytes. We observed

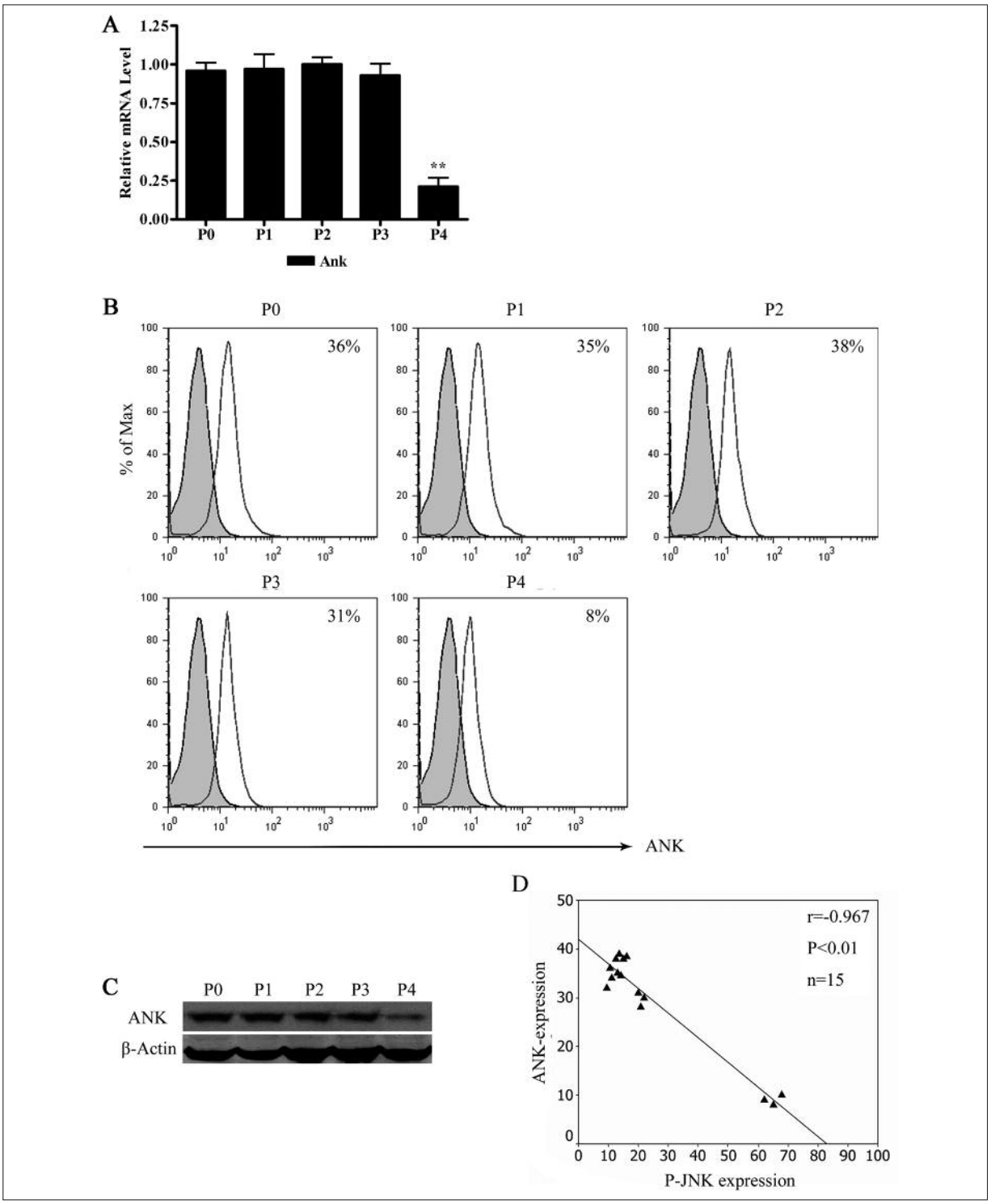

Figure 3. ANK expression in naturally degenerated rat endplate chondrocytes. Endplate chondrocytes were grown to confluency, trypsinized and repetitively subcultured into new dishes until the cells reached passage 4. ***A,*** The ANK mRNA expression levels in the P0-P4 rat endplate chondrocytes were analyzed by qPCR. ***B,*** The ANK protein expression levels in the P0-P4 endplate chondrocytes were analyzed by flow cytometry. ***C,*** The ANK protein expression levels in the P0-P4 endplate chondrocytes were analyzed by Western blot. ***D,*** The ANK protein expression levels negatively correlate with JNK phosphorylation in the P0-P4 endplate chondrocytes ($r = -0.967$, $p < 0.01$). Results were obtained in 3 independent experiments, and representative results are presented in panels B and C. Data are shown as mean ± SD. $^{**}p < 0.01$ vs. primary cells. P0: primary cells; P1-P4: passage 1-4 cells.

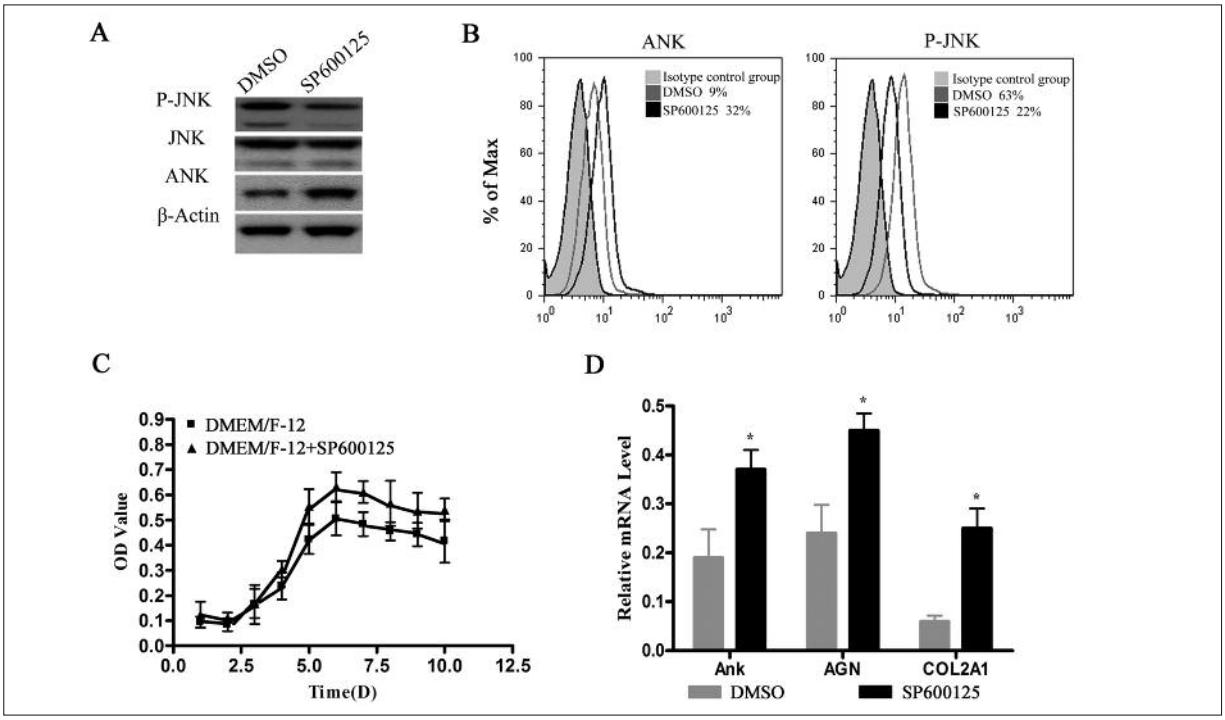

Figure 4. Effect of inhibition of the JNK pathway on mRNA expression levels of ANK and other genes in the passage 4 rat endplate chondrocytes. ***A,*** The passage 4 (P4) rat endplate chondrocytes were incubated with or without JNK inhibitor SP600125 (10 μM for 1 hour). The expression levels of p-JNK and total ANK in the P4 endplate chondrocytes were analyzed by Western blot. ***B,*** The P4 rat endplate chondrocytes were cultured as above, with or without SP600125. The expression levels of p-JNK and total ANK in the P4 endplate chondrocytes were analyzed by flow cytometry. ***C,*** The P4 endplate chondrocytes were cultured for 11 days with or without SP600125 (10 μM), and cell absorbances were plotted. D, The P4 rat endplate chondrocytes were cultured for 1 hour with or without SP600125 (10 μM). The mRNA expression levels of ANK, type II collagen and aggrecan in the P4 rat endplate chondrocytes were analyzed by qPCR. Results were obtained in 3 independent experiments, and representative results are presented in panels 4A, 4B, and 4D. Data are shown as mean ± SD. *$p < 0.05$ vs. cells without SP600125 treatment.

that JNK phosphorylation levels increase during the course of passaging, with the most significant increase observed in the passage 4 cells.

SP600125, a reversible ATP competitive inhibitor, dose-dependently inhibits phosphorylation of c-Jun and, by that, phosphorylation of downstream target proteins of JNK[35]. To determine whether activation of JNK is directly associated with endplate chondrocytes degeneration, we inhibited the JNK activity using SP600125. Our results show that this inhibition attenuated natural degeneration in passage 4 endplate chondrocytes. These results are in line with previous observations[36-38]. Clancy et al[36] and Fan et al[37] found that phosphorylation levels of JNK are higher in the osteoarthritis cartilage. SP600125 is able to inhibit increased metalloproteinases 13 (MMP-13) production in articular chondrocytes[36]. MMP-13 plays an important role in cartilage matrix degradation. Moreover, Kang et al[38] found that adiponection could cause matrix degradation in the osteoarthritis cartilage and increase MMPs and iNOS expression via the AMP-activated protein kinase (AMPK) and JNK pathways in human osteoarthritis chondrocytes.

The protein kinase B (AKT) signaling pathway plays critical role in chondrocytes growth, survival and terminal differentiation[17-24]. Besides, recent studies found that the AKT signaling pathway might participate in the progress of IVD degeneration[39]. We, therefore, sought to determine changes in the AKT phosphorylation in the process of degeneration of cervical endplate chondrocytes. Our findings showed that phosphorylation levels of AKT did not change during cell degeneration. Therefore, we did not follow up with blocking of AKT signaling pathway. These results of our study contradict previous observations, possibly because of differing origins of chondrocytes.

Studies indicate that the ANK-mediated control of pyrophosphate levels may be a potential mechanism regulating tissue calcification, in-

cluding cartilage tissue[26]. Change in ANK expression may contribute to the loss of phenotype observed in articular chondrocytes under pathological conditions[40]. It was previously shown that both mRNA and protein levels of ANK are lower in degenerated cervical endplate compared with normal cervical endplate[28]. Further, ANK gene is down-regulated during intermittent cyclic mechanical tension induced by the calcification of endplate chondrocyte[41]. In the present study, we found that ANK expression, both mRNA and protein, was down-regulated during degeneration of endplate chondrocytes. These findings are in a good agreement with previous observations[28,29,42] indicating that ANK may play a role in the process of degeneration of endplate chondrocytes.

Recently, several works reported relationship between the ANK and ERK, PKC, and Wnt signaling pathways in pathophysiological processes in chondrocytes[40,43]. However, no data are available on the relative contributions of the ANK and JNK signaling pathways to degeneration of chondrocytes. Our results show that levels of phosphorylated JNK significantly and negatively correlate with ANK expression during natural degeneration of rat endplate chondrocytes. Previously, Cailotto et al[43] found that TGF-beta1 increases ePPi levels, mainly by induction of the ANK gene which requires activation of Ras, Raf-1, ERK, and Ca^{2+}-dependent PKC pathways in chondrocytes. Therefore, our results indicate that phosphorylation of JNK promotes natural degeneration of cervical endplate chondrocytes, likely by down-regulating ANK expression.

Conclusions

In the present study, we found that phosphorylation of JNK, but not AKT, promotes natural degeneration of cervical endplate chondrocytes, likely by down-regulating ANK expression. This implicates that inhibition of JNK signaling pathway may halt the natural degeneration of cervical endplate chondrocytes. This pathway, thus, is a potential new target to prevent the IVD degeneration.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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