European Review for Medical and Pharmacological Sciences 2014; 18: 2835-2845

The roles of MAPKs in rabbit nucleus pulposus cell apoptosis induced by high osmolality

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Abstract. – BACKGROUND: Earlier work has suggested that the p38 MAPK, JNK1/2 and ERK1/2 signal pathway existed in nucleus pulposus cells and the cell growth, differentiation and apoptosis were regulated by them. Because osmotic fluctuations are inevitable in the physicochemical environment of intervertebral disc cells, high osmolality could activate p38 MAPK, JNK1/2 and ERK1/2 signal pathway. The effects of high osmolality on the catabolic program and proliferation of nucleus pulposus cells are still not clear.

AIM: To explore the possible roles of MAPKs in rabbit nucleus pulposus cell apoptosis induced by high osmolality.

MATERIALS AND METHODS: Rabbit nucleus pulposus cells were cultured and divided into different group at random. The cells were pretreated with inhibitor for p38 MAPK, JNK1/2 and ERK1/2 signal pathway respectively. In next step, the cells were cultured in different osmolality environment for different time at 37°C in 5% carbon dioxide incubator. After treatments, ratio of apoptosis was measured by flow cytometry, and western blotting was performed to quantify the expression of the activated forms of p38 MAPK, JNK1/2 and ERK1/2. Furthermore, immunofluorescence analysis with confocal microscopy was performed to confirm the hyperosmolality effects on activation of p38 MAPK, JNK1/2 and ERK1/2 signal pathways in nucleus pulposus cells.

RESULTS: Our results show that in 500 and 600 mOsm/kg medium, rabbit nucleus pulposus cell apoptosis increased, and a persistent phosphorylation of p38 MAPK, JNK1/2 and ERK1/2 proteins were observed. In the same condition, the apoptotic cells death remarkably decreased when the p38 MAPK and JNK1/2 signal pathways were blocked by their inhibitors SB203580, SP600125 repectively. On the other side, the apoptotic cells death rate reraised greatly when the ERK1/2 signal pathways were blocked by its inhibitor PD98059.

CONCLUSIONS: High osmolality activated p38MAPK, JNK1/2 and ERK1/2 in rabbit nucleus pulposus cell, and the activated p38 MAPK and JNK1/2 induced cell apoptosis, on the contrary, the activated ERK1/2 made the cell survived.

Key Words:
Osmotic pressure, p38 MAPK, JNK1/2, ERK1/2, Apoptosis.

Introduction

Nucleus pulposus cell apoptosis is one of the changes of intervertebral disc degeneration (IVD)[1]. The nucleus pulposus is an avascular and hydrated tissue that provides a special environment for nucleus pulposus cells. As the nucleus pulposus tissue is an osmotic system, its hydration is not constant but varies with applied loads and activities. *In vivo* loading is applied mainly by body weight and muscle activity. Loads are lowest at night during rest (typically 0.1-0.2 Mpa). During the day's activities, intradiscal pressures can rise to 3 MPa. As a result, fluid is expressed from the IVD during day (up to 25% of fluid loss) and re-imbibed at night when the loads are reduced[2,3]. Consequently, as a result of this diurnal change of fluid, the extracellular osmolarity changes proportionately. In some previous studies, changes in aggrecan, collagen I and collagen II expression of IVD cells were examined after exposure to osmotic environment alterations or mechanical stimulation under different osmotic conditions. Changes in gene expression of IVD cells induced by osmolarity variation were also measured; and some gene expressiones related to signal pathway protein[4-6].

Members of the mitogen-activated protein kinases (MAPKs) family regulate cell growth, differentiation and apoptosis. Three subfamilies have been identified in mammalian cells: extracellular signal-regulated kinase (ERK1/2), p38 mitogen-activated protein kinase (p38 MAPK, p38) and c-Jun amino terminal kinase (JNK1/2). Apart from

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MAPKs, the signaling pathway composed of type I insulin-like growth factor receptor (IGFR), phosphatidylinositol 3′-kinase (PI3K), protein kinaseB (PKB/Akt) and mammalian target of rapamycin (mTOR), is crucial for cell growth, proliferation and survival[7]. In addition, mTOR is one of fundamental regulators for cell proliferation and differentiation[8]. P38 and JNK1/2 are serine and threonine protein kinases that are activated by various stress stimuli, including, osmotic shock, toxic compounds and proinflammatory cytokines, which may regulate cell growth, differentiation and apoptosis[9]. In a previous study, *in vitro* ERK1/2 can be activated by high osmolality in nucleus pulposus cells[10]. Although many MAPK-activating stimuli are proapoptotic or antiapoptotic, the biological effects of MAPK activation is highly divergent and appears to be largely dependent on the cell type[11]. However, the intracellular signaling pathways involved in the hyperosmotic stress-induced nucleus pulposus cell apoptosis are not yet clear.

Therefore, the aim of the present work was to investigate the influence of high osmolality on the nucleus pulposus cell viability by means of flow cytometry assay and to study the changes of the apoptotic signaling molecules possibly involved by means of western blot.

Materials and Methods

Materials

JNK1/2, p38 MAPK, ERK1, ERK2, phospho-p38 MAPK, SP600125, SB203580 PD98059, Fluorescein isothiocyanate (FITC)-labeled annexin V, Propidium iodide (PI), Goat anti Rabbit or Mouse IgG horseradish peroxidase-conjugated (IgG-HRP), FITC-conjugated Goat Anti-mouse IgG (IgG-FITC) and RBITC-conjugated Goat Anti Rabbit IgG (IgG-RBITC) were from Beyotime (Suzhou, China). phospho-ERK1/2 (Thr202/Tyr204), phospho-JNK1/2 (Thr183/Tyr185) were from Cell Signaling (Beverly, MA, USA).

Cell Culture

All animal and cell studies have been approved by China Ethics Committee and performed in accordance with the ethical standards. The nucleus pulposus cells were isolated from 1-month-old New Zealand rabbit using an explant culture methodology reported previously[4]. Briefly, rabbits were sacrificed by intramuscular injection with 0.5% pentobarbital sodium (0.1 mg/kg). The spinal columns were removed en bloc under aseptic conditions, and lumbar intervertebral discs were collected. Nucleus pulposus cells were separated from the nucleus tissue by 0.25% collagenase II (Sigma, St Louis, MO, USA) for 20-30 minutes at 37°C, and cells were cultured with Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham's, 1:1 Mixture (DMEM/ F-12) (Hyclone, Waltham, MA, USA) that was supplemented with 10% fetal bovine serum (Sijiqing, Hangzhou, China) at 37°C in 5% carbon dioxide incubator. When confluent, the cells were lifted using trypsin (0.25%) solution contained EDTA (1 mmol/L) (Sigma) and subcultured on 25 cm^2 culture flask. There are no significant changes in cell morphology between freshly isolated cells (passage 0) and later passage (passage 5). Cells also did not exhibit a spindle shaped, fibroblastic phenotype during passaging. Therefore, cells from passages 3 to 7 were used for the studies.

JNK1/2 inhibitor SP600125, p38 MAPK inhibitor SB203580 and ERK1/2 inhibitor PD98059 were used at final concentrations of 10 μM, 10 μM and 50 μM respectively. In SP600125 inhibitor groups, the cells were pretreated with 10 μM SP600125 for 30 minutes, and SB203580 inhibitor groups with 10 μM SB203580 for 30 minutes also; In PD98059 inhibitor groups, the cells were pretreated with 50 μM PD98059 for 1 hour; and then cells were treated with different osmolality for different time at 37°C in 5% carbon dioxide incubator.

300 mOsm, 500 mOsm and 600 mOsm medium were adjusted by the addition of NaCl as previously described[12,] and for all solutions the osmolality of all solutions was measured by freezing-point depression with automatic cryoscopic osmometer (model PM-8P, China). For all conditions, the pH was maintained at 7.4±0.2.

Flow Cytometric Assay of Apoptosis

Ratio of apoptosis was measured by flow cytometry after double staining with FITC-labeled annexin V and PI, and also PI secondary dye is used to differentiate apoptotic cells from viable and necrotic cells[13]. Cells were cultured with or without MAPK inhibitors for different time (3 h-6 h) under different osmolality medium (300 mOsm-600 mOsm). First, the attached cells were harvested (5 mmol/L EDTA) and pooled with floating (detached) cells. Cells were then collected by centrifugation (5 minutes, 200 g) and resuspended in phosphate buffered saline (PBS).

10^5 cells were harvested and 185 μL of annexin binding buffer (Beyotime, Suzhou, China) was added to tube as described by the manufacturers, then incubated for 15 minutes with 5 μL FITC-annexin V and 10 μL PI at 25°C away from light. The samples were immediately measured by flow cytometry (Becton, Dickinson Inc., Franklin Lakes, NJ, USA).

Western Blot Analysis

The cells were rinsed with ice-cold PBS, and then harvested in 100 μL radio immunoprecipitation assay lysis buffer [50 mmol/L Tris-HCl pH 7.5, 0.15 M NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), RIPA] with 1 mmol/L phenylmethanesulfonyl fluoride (PMSF) on ice for 30 minutes. Lysates were centrifuged at 4°C for 10 minutes at 12,000 g and resolved on 10% SDS- polyacrylamide gels (SDS-PAGE). Proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Beyotime, Suzhou, China) by electroblotting. The membranes were blocked with 5% nonfat milk in Tris Buffered Saline (TBS) (50 mmol/L Tris, pH 7.6, 150 mmol/L NaCl, 0.1%) and incubated overnight at 4°C in 3% nonfat dry milk in TBS-Tween (0.2% Tween, TBST) with appropriate primary antibodies. Membranes were washed three times for 5 minutes in TBST and incubated with a appropriate IgG-HRP for 1h at room temperature. The detections were performed using electrochemiluminescence detection reagent (Beyotime, Suzhou, China). Equal amounts of protein (30 μg) were electrophoresed on 10% SDS-PAGE. The protein bands were transferred to PVDF membrane and reacted with the primary antibody and detected by electrochemiluminescence detection. Grey level was analyzed by Gel-Pro analyzer software.

Immunofluorescence

Nucleus pulposus cells cultured on glass-coverslips were fixed with 3.7% (v/v) formaldehyde for 15 minutes at room temperature, permeabilized with 0.2% (v/v) Triton X-100 in PBS for 5 minutes on ice, blocked with bovine serum albumin (BSA) (Sijiqing, Hangzhou, China) 1% (w/v) for 1h at room temperature and incubated overnight at 4°C with appropriate primary antibody. Following washing in TBST, the cells were incubated with IgG-RBITC and IgG-FITC for 1h and nuclei were counter-stained with 4',6-diamidino-2-phenylindole (DAPI) at a final concentration of 2 μg/ml. Labeled cells were visualized using a confocal laser scanning microscopy.

Statistical Analysis

Data presented were the mean of at least three independent experiments (±standard deviation). Differences were considered significantly when $p < 0.05$ (Student's t test for two samples comparison and ANOVA, Tukey's test for multiple samples comparison). All analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) with values for $p < 0.05$ being regarded as significant.

Results

Hyperosmosis Induces Nucleus Pulposus Cells Apoptosis

By flow cytometric assay of apoptosis, we found that the control group had more than 90% of intact, living cells and only less than 5% of cells in the early and late phases of apoptosis. The nucleus pulposus cells in 300 mOsm medium with or without inhibitors for 3 hours (h), 6 h, 12 h respectively showed no significant difference in the percentage of living and apoptotic cells compared to the control group. In 500 mOsm medium (for 3 h, 6 h, 12 h respectively), cell apoptosis ratio increased from 8.3% to 11.5%, but cell apoptosis ratio decreased and fluctuated between 5.3% and 8.1% in SB203580 inhibitor group, and between 5.1% and 8.2% in SP600125 group. In contrast, cell apoptosis ratio increased from 8.9% to 15.5% after pretreated with PD98059. A marked increase of apoptotic cells from 19.9% to 43.5% was observed in the 600 mOsm medium. But, pretreated with SP600125 and then treated with 600 mOsm medium, apoptotic cells decreased apparently and fluctuated between 4.1% and 10.4%. SB203580+600 mOsm medium group decreased between 4.3% and 10.9%. However, interestingly pretreated with PD98059 and then treated with 600 mOsm medium, apoptotic cells magnify from 30.5% to 70.5% (Figure 1). Since no differences were found between viability of control cells and those exposed to 300 mOsm medium, signaling pathways protein were examined only in cells exposed to 600 and 500 mOsm medium.

Expression of p38 MAPK and Phospho-p38 MAPK in Nucleus Pulposus Cells

We examined the activity of the MAPK pathway in nucleus pulposus cells by western blot. As shown in Figure 2,3, the bands of p38 MAPK could be identified in the seven groups

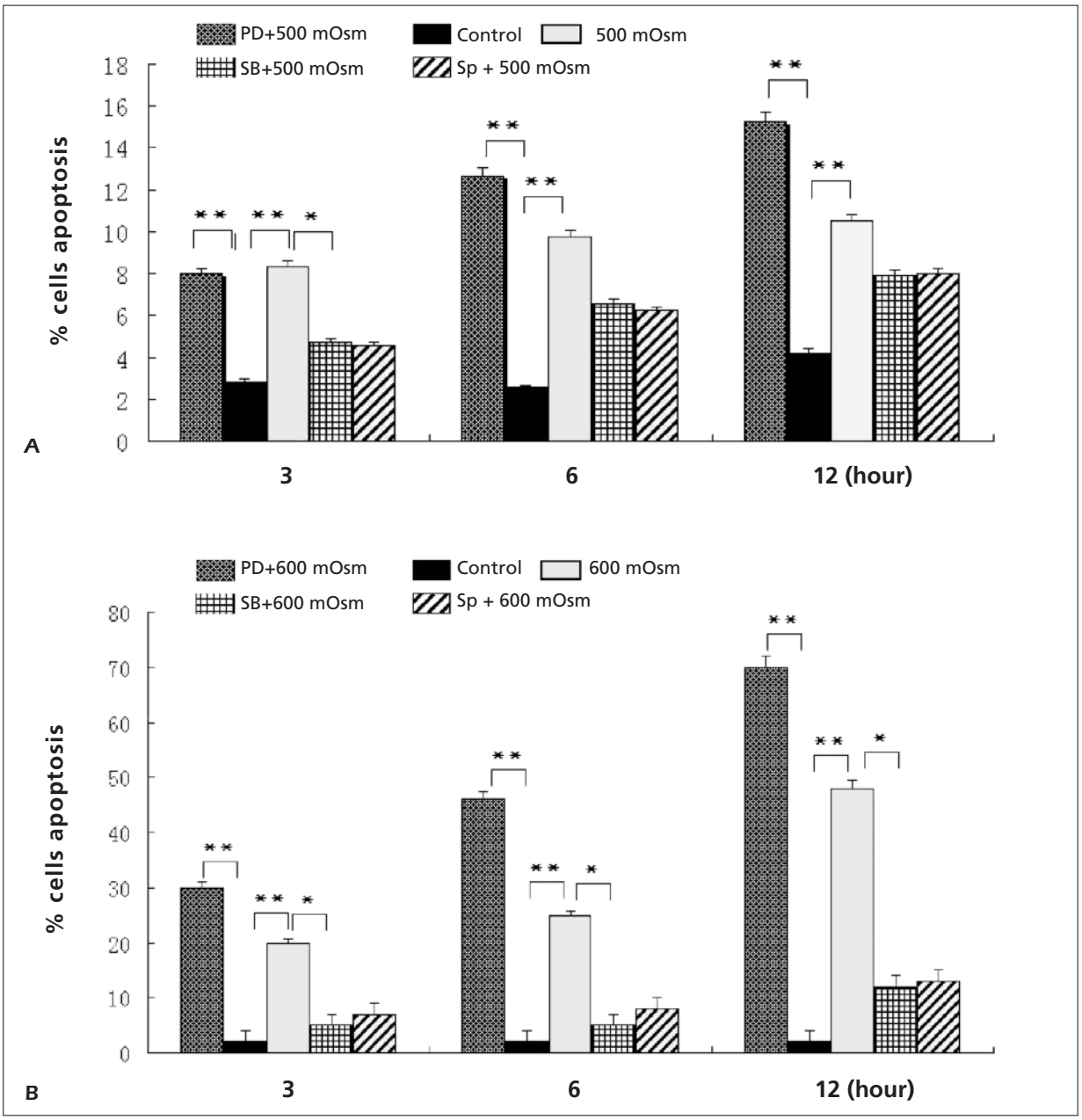

Figure 1. Percentage of cells in early and late phase of apoptosis. In 300 mOsm medium, cell apoptosis ratio was less than 5%. ***A,*** In 600 mOsm medium, cell apoptosis ratio increased from 19.9% to 43.5% with treatment time prolong, but cell apoptosis ratio decreased and fluctuated between 4.1% and 10.4% in SB203580 group and SB203580 group decreased between 4.3% and 10.9%. In contrast, cell apoptosis ratio increased from 30.5% to 70.5% with treatment time prolong after pretreated with PD98059. ***B,*** In 500 mOsm medium, cell apoptosis ratio increased from 8.3% to 11.5%, but cell apoptosis ratio decreased and fluctuated between 5.3% and 8.1% in SB203580 group, but fluctuated between 5.1% and 8.2% in SP600125 group. In contrast, cell apoptosis ratio increased from 8.9% to 15.5% after pretreated with PD98059.*$p < 0.05$; **$p < 0.01$.

and the density of the bands of the seven groups seemed similar. phospho-p38 protein showed a more than 3-fold increase in nucleus pulposus cells subjected to 600 and 500 mOsm medium compared to control levels; with treatment time prolong, the expression of phospho-p38 protein decrease gradually. There was less phospho-p38 protein being detected in nucleus pulposus cells pretreated by SB203580 under the same conditions.

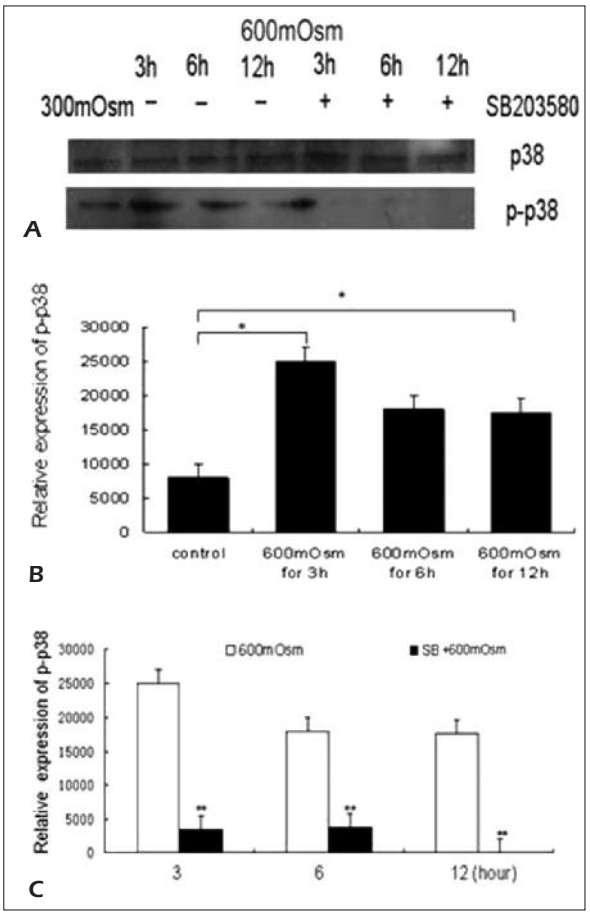

Figure 2. The expression of phospho-p38 protein in nucleus pulposus cells cultured in 600 mOsm medium. ***A,*** Grey level was analyzed by Gel-Pro analyzer software and standardized. ***B,*** p38 protein in nucleus pulposus cells cultured in 600 mOsm medium for 3 hours was activated remarkably, and decreased gradually with time prolong. ***C,*** But phospho-p38 protein in cells was less expressed with SB203580. *$p < 0.05$; **$p < 0.01$.

Expression of JNK1/2 and Phospho-JNK1/2 in Nucleus Pulposus Cells

Phospho-JNK1/2 protein was expressed in nucleus pulposus cells treated in 300 mOsm medium, while expression of phospho-JNK1/2 in 600 mOsm medium showed an even more marked, an almost 10-25 fold increase compared to control levels (Figure 4). And the expression of phospho-JNK1/2 in 500 mOsm medium showed a greaat increase compared to control levels (Figure 5). With treatment time prolong, the expression of phospho-JNK1/2 decrease gradually. After pretreated nucleus pulposus cells with SP600125, there were almost no phospho-JNK1/2 expression being detected in nucleus pulposus cells in 600 and 500 mOsm medium.

Expression of ERK1/2 and Phospho-ERK1/2 in Nucleus Pulposus Cells

Expression of ERK1/2 protein can be identified in the seven groups and the density of the bands of the seven groups seem similar. Phospho-ERK1/2 in nucleus pulposus cells under 600 mOsm medium showed a more than 10 fold increase compared to control levels (Figure 6). And the expression of phospho-ERK1/2 in 500 mOsm medium showed a marked increase compared to control levels (Figure 7). With treatment time prolong, the expression of phospho-ERK1/2 protein decrease gradually. Less expression of phospho-ERK1/2 protein was detected in nucleus pulposus cells pretreated by PD98059 under 600 mOsm medium.

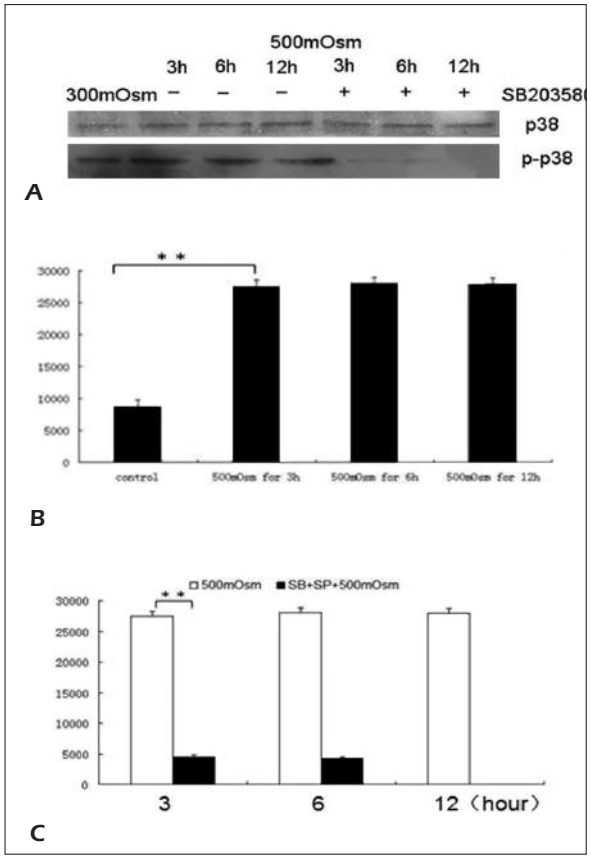

Figure 3. The expression of phospho-p38 protein in nucleus pulposus cells cultured in 500 mOsm medium. ***A,*** Grey level was analyzed by Gel-Pro analyzer software and standardized. ***B,*** p38 protein in nucleus pulposus cells cultured in 500 mOsm medium for 3 hours was activated, and decreased gradually with time prolong. ***C,*** But phospho-p38 protein in cells was less expressed with SB203580. *$p < 0.05$; **$p < 0.01$.

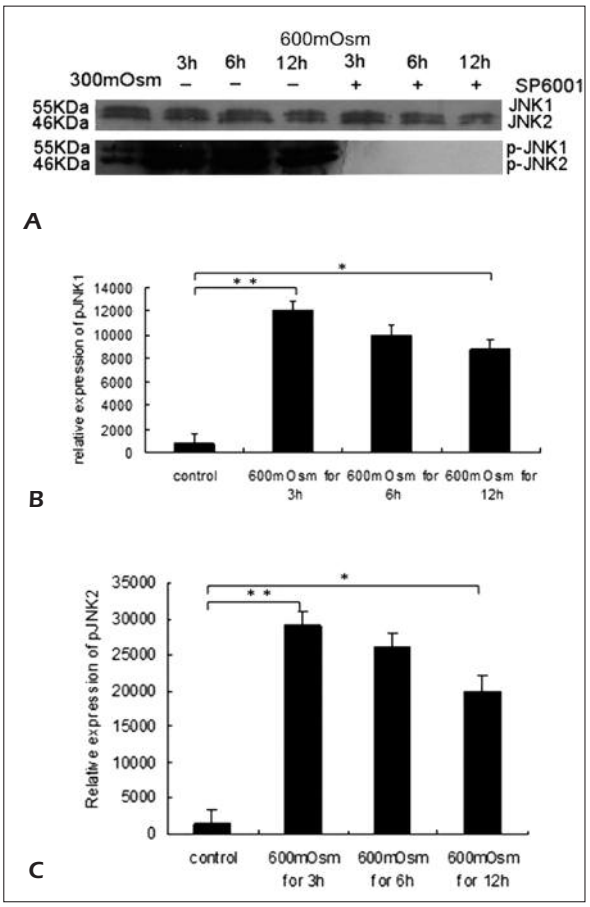

Figure 4. The expression of phospho-JNK1/2 protein in nucleus pulposus cells cultured in 600 mOsm medium. ***A,*** Grey level was analyzed by Gel-Pro analyzer software and standardized. ***B,*** JNK1 protein in nucleus pulposus cells cultured in 600 mOsm medium for 3 hours was activated remarkably, and decreased gradually with time prolong. But phospho-JNK1 protein in cells was not expressed with SP600125 under the same condition. ***C,*** Likewise phospho-JNK1 protein, JNK2 protein in nucleus pulposus cells was activated remarkably, and phospho-JNK1 protein in cells was not expressed with SP600125. *$p < 0.05$; **$p < 0.01$.

Immunofluorescence Analysis of Phospho-p38 MAPK, Phospho-JNK1/2 and Phospho-ERK1/2 in Nucleus Pulposus Cells

To confirm the hyperosmolality effects on activation of p38 MAPK, JNK1/2 and ERK1/2 signal pathways in nucleus pulposus cells, we further investigated phospho-p38 MAPK, phospho-JNK1/2 and phospho-ERK1/2 by conducting immunofluorescence analysis using confocal microscopy. Similar to the western blot analysis, it was found that the phospho-p38 MAPK, phospho-JNK1/2 (Figure 8) and phospho-ERK1/2 (Figure 9) in cells treated by 600 and 500 mOsm medium for 3h were expressed significantly.

Discussion

Osmotic fluctuations are inevitable in the physicochemical environment of intervertebral disc cells, as variations in disc mechanical loading lead to significant changes in tissue hydration. However, even though osmotic pressure is a considerable feature of disc cell's microenvironment, very few studies have addressed the impact of this type of stress at the cellular level[14-16]. In our study, the results indicated that hyperosmotic stress leads mainly to apoptotic cell death that involves changes in the apoptotic signaling molecules in a primary cultured nucleus pulposus cells. Hyperosmotic environment (500 and 600 mOsm) induced the activation of proapoptotic signaling factors such as JNK1/2, p38 MAPK and also led to ERK1/2 increase. However, slight osmotic pressure increase (300

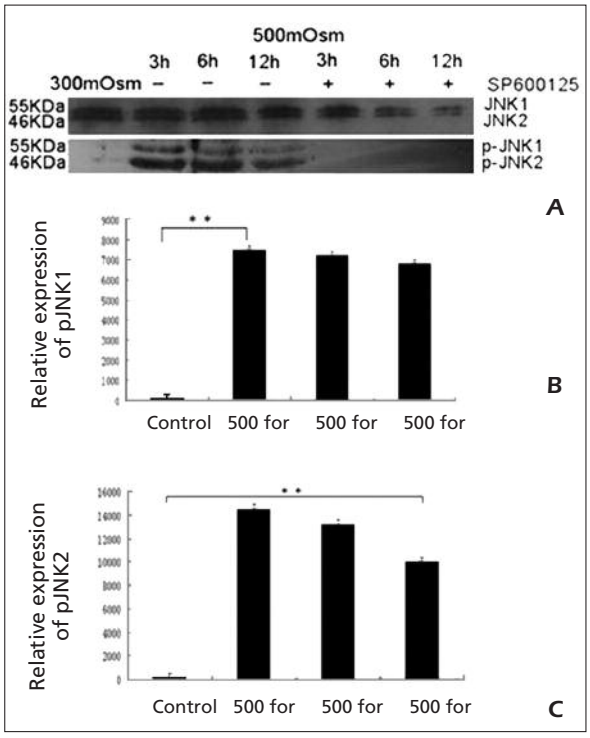

Figure 5. The expression of phospho-JNK1/2 protein in nucleus pulposus cells cultured in 500 mOsm medium. ***A,*** Grey level was analyzed by Gel-Pro analyzer software and standardized. ***B,*** JNK1 protein in nucleus pulposus cells cultured in 500 mOsm medium for 3 hours was activated remarkably, and decreased gradually with time prolong. But phospho-JNK1 protein in cells was not expressed with SP600125 under the same condition. ***C,*** Likewise phospho-JNK1 protein, JNK2 protein in nucleus pulposus cells was activated remarkably, and phospho-JNK1 protein in cells was not expressed with SP600125. *$p < 0.05$; **$p < 0.01$.

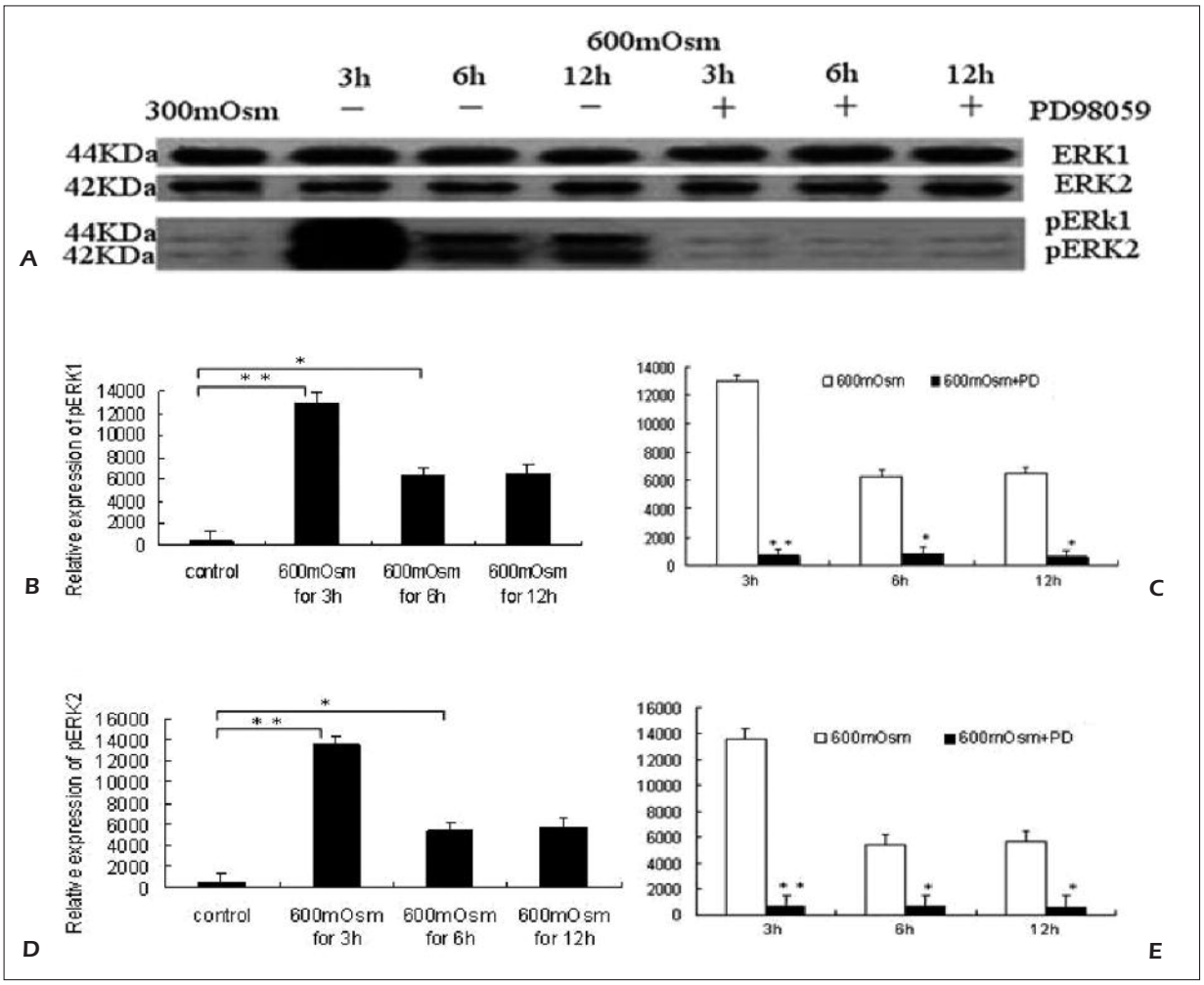

Figure 6. The expression of phospho-ERK1/2 protein in nucleus pulposus cells cultured in 600 mOsm medium. ***A,*** Grey level was analyzed by Gel-Pro analyzer software and standardized. ***B,*** ERK1 protein in nucleus pulposus cells cultured in 600 mOsm medium for 3 hours was activated remarkably, and decreased gradually with time prolong. ***C,*** But phospho-ERK1 protein in cells was less expressed with PD98059 under the same condition. ***D,*** Likewise phospho-ERK1 protein, ERK2 protein in nucleus pulposus cells was activated remarkably. ***E,*** phospho-ERK2 protein was less expressed with PD98059. $*p < 0.05$; $**p < 0.01$.

mOsm) could not lead to the changes of nucleus pulposus cells and could not induce cell apoptosis, which indicate nucleus pulposus cells can adapt to gentle osmotic pressure increase. This adaptability may be related to Ton EBP/ OREBP, ASIC3 and ERK pathway[14,17,18]. The nucleus pulposus cells shrinked and the volume decreased after treatment with 500 and 600 mOsm medium. In our research, it was showed that a slight elevation of osmotic pressure within the physiological range did not cause any changes affecting cell survival but the cell apoptosis happened when nucleus pulposus cells subjected to a severe hyperosmotic medium, which is in accordance with previous study[15].

The increase of osmotic pressure could activate JNK1/2, p38 MAPK pathways and then regulate cell metabolism[9]. And then the activated JNK1/2, p38 MAPK can start the apoptosistic procedure that induce apoptosis by transcription factors[19-21]. There are JNK1/2, p38 MAPK and ERK1/2 pathways in nucleus pulposus cells, which to a larger extent regulate nucleus pulposus cell metabolism[10,22]. In this study, we observed the expression of phospho-JNK1/2, phospho-p38 and phospho-ERK1/2 in cytoplasm and nucleus of nucleus pulposus cells treated by hyperosmolality. Furthermore, there was less expression of phospho-JNK1/2, phospho-p38 and phospho-ERK1/2 in normal nucleus pulposus

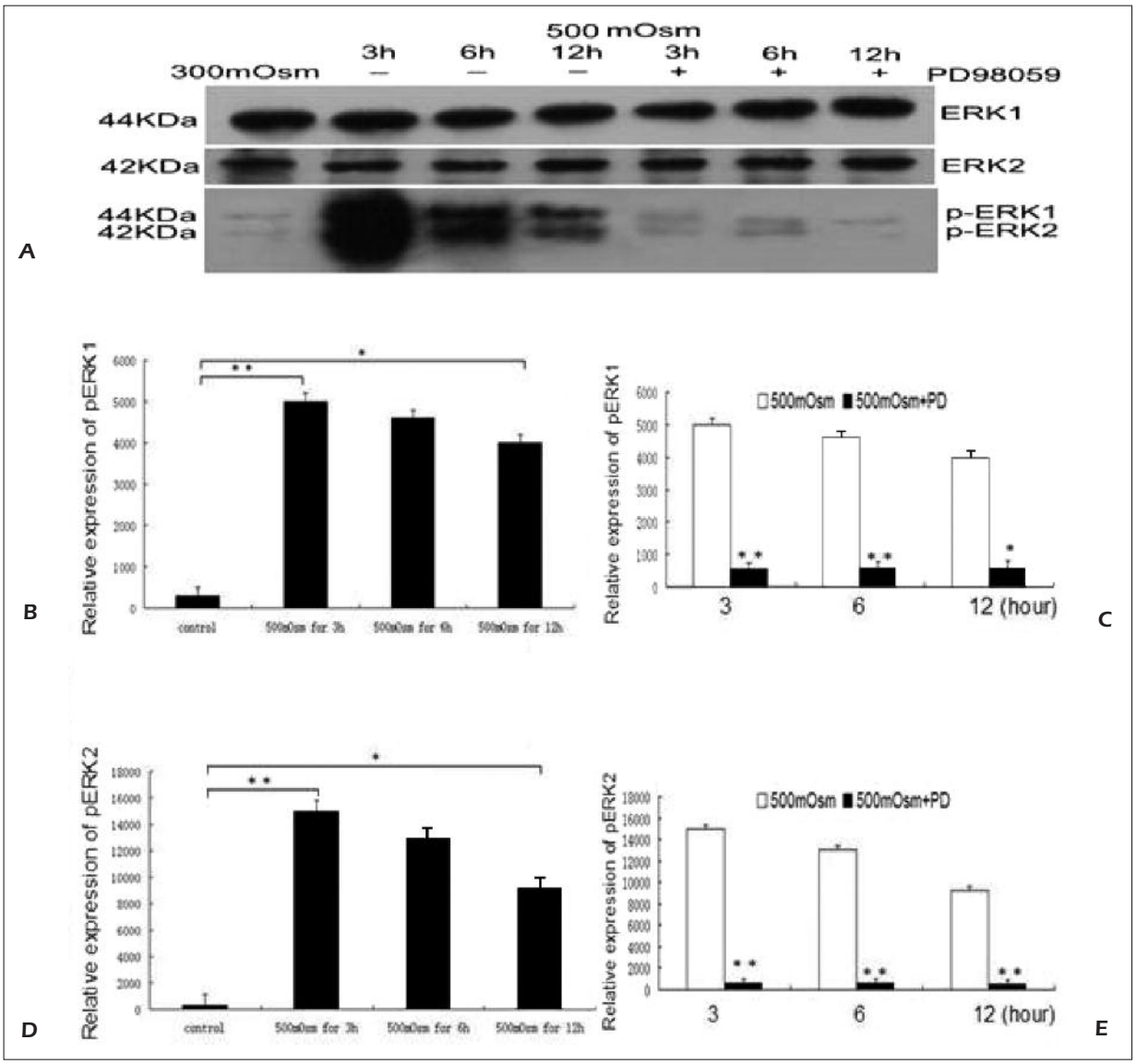

Figure 7. The expression of phospho-ERK1/2 protein in nucleus pulposus cells cultured in 500 mOsm medium. ***A,*** Grey level was analyzed by Gel-Pro analyzer software and standardized. ***B,*** ERK1 protein in nucleus pulposus cells cultured in 500 mOsm medium for 3 hours was activated remarkably, and decreased gradually with time prolong. ***C,*** But phospho-ERK1 protein in cells was less expressed with PD98059 under the same condition. ***D,*** Likewise phospho-ERK1 protein, ERK2 protein in nucleus pulposus cells was activated remarkably. ***E,*** phospho-ERK2 protein was less expressed with PD98059. $*p < 0.05$; $**p < 0.01$.

cells, but the expression increased when the nucleus pulposus cells were exposed to a high osmotic pressure. However, when the treatment time was prolonged, the expression of phospho-JNK1/2, phospho-p38 and phospho-ERK1/2 decreased gradually. Recently study demonstrated that Cadmium (Cd) induced neuronal apoptosis in part by activation of the MAPK and mTOR pathways; In addition, Cd elevated intracellular calcium ion ($[Ca^{2+}]i$) level in PC12, SH-SY5Y cells and primary murine neurons, which induced reactive oxygen species (ROS) and activated MAPK and mTOR pathways, leading to neuronal apoptosis[23]. In present study, hyperosmolality can activate MAPK pathways and the expression of phospho-JNK1/2, phospho-p38 and phospho-ERK1/2 decreased gradually with the treatment time prolong, which may be related to $Ca^{2+}]i$ level in nucleus pulposus cells.

SB203580 is the special antagonist of p38, which affect Thr106 the active spot of p38 and then block p38 signal pathway[24,25]. SP600125 is

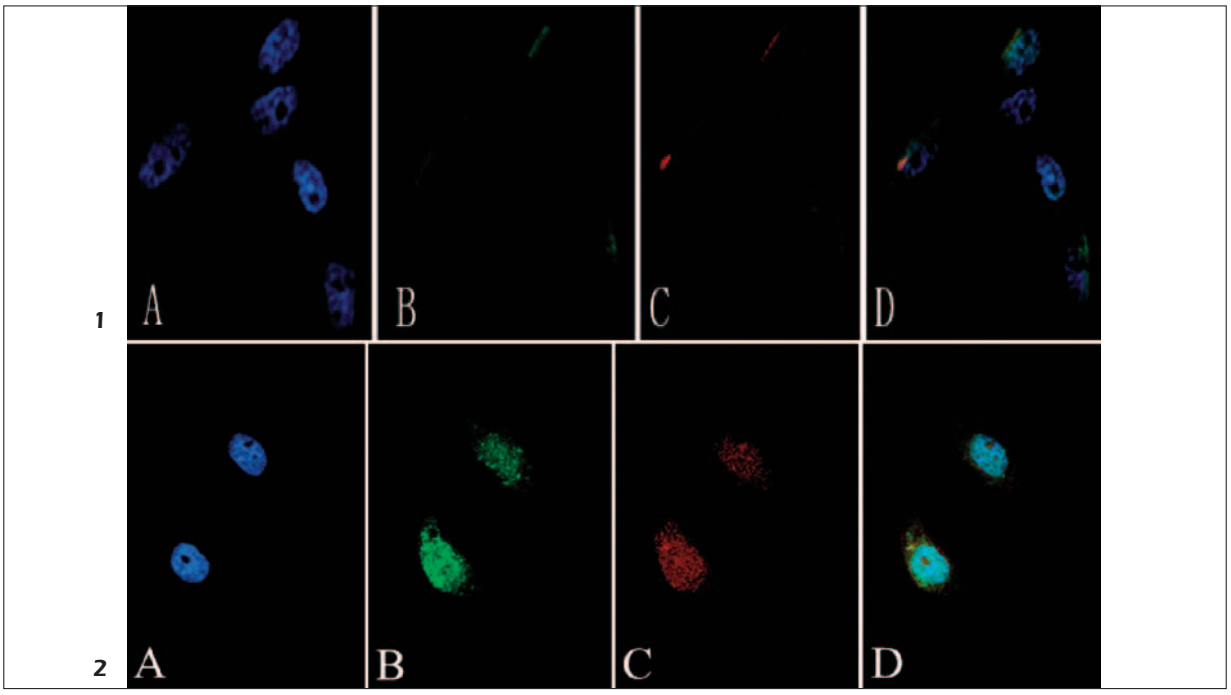

Figure 8. The distribution and expression of phospho-JNK1/2 and phospho-p38 MAPK in nucleus pulposus cells (×200). ***1,*** Nucleus pulposus cells cultured in 300 mOsm medium. ***2,*** Nucleus pulposus cells treated with 600 mOsm medium for 3h. **A,** Regions stained by DAPI revealing the nuclear structure of the cells. **B,** The expression of phospho-JNK1/2 in cytoplasm and nucleus. **C,** The expression of phospho-p38 in cytoplasm and nucleus. **D,** Confocal image of above images.

the antagonist of JNK1/2, which can prevent JNK1/2 from activating by blocking ATP active spot of JNK1/2[26-28]. Two types of antagonists which could block p38 and JNK1/2 signal pathways were used in our research to detect the involvement of the possible signal pathways. Previous study indicate that p38 inhibition in nucleus pulposus cells can prevent intervertebral disc degeneration[29]. In our study, even though the nucleus pulposus cells were exposed to hyperosmotic medium, the expression of phospho-p38 was decreased when p38 signal pathway was inhibited by SB203580, and we found the same phenomenon in the JNK1/2 group; on the same time, the apoptosis of nucleus pulposus cells both decrease effectively. Thus, the p38, JNK1/2 aignal pathways involved in the nucleus pulposus cells apoptosis caused by hyperosmolaity. The activation of ERK1/2 signal pathway is mostly linked with growth factor action, cellular protection and proliferation[30-31]. And recently study showed that curcumin rapidly induced activation of the MAPKs including ERK1/2 and JNK and induced p53-independent apoptosis; at the same time curcumin induction of ROS activates MAPKs, at least partially by inhibiting protein phosphatases 2A (PP2A) and 5 (PP5)[32]. PD98059 is a specific antagonist of the activation of mitogen-activated protein kinase *in vitro* and *in vivo*[33]. The ERK1/2 protein was also activated under hyperosmotic conditions. When ERK1/2 signal pathway was inhibited by PD98059 the expression of phospho-ERK1/2 was decreased, and the apoptosis rate of nucleus pulposus cells increased effectively. All these findings indicated that p38 and JNK1/2 participated in the induction of the catabolic program of nucleus pulposus cells, which lead to nucleus pulposus cell apoptosis; ERK1/2 participated in proliferation of nucleus pulposus cells.

Conclusions

This study showed that a slight hyperosmotic stress induced no changes in cell viability, while a highly hyperosmotic medium led to profound changes in primary nucleus pulposus cell culture. Hyperosmotic stress induced apoptotic cell death in nucleus pulposus as revealed by FITC-labeled annexin V and PI double-staining. It also induced phosphorylation of members of the MAPK fami-

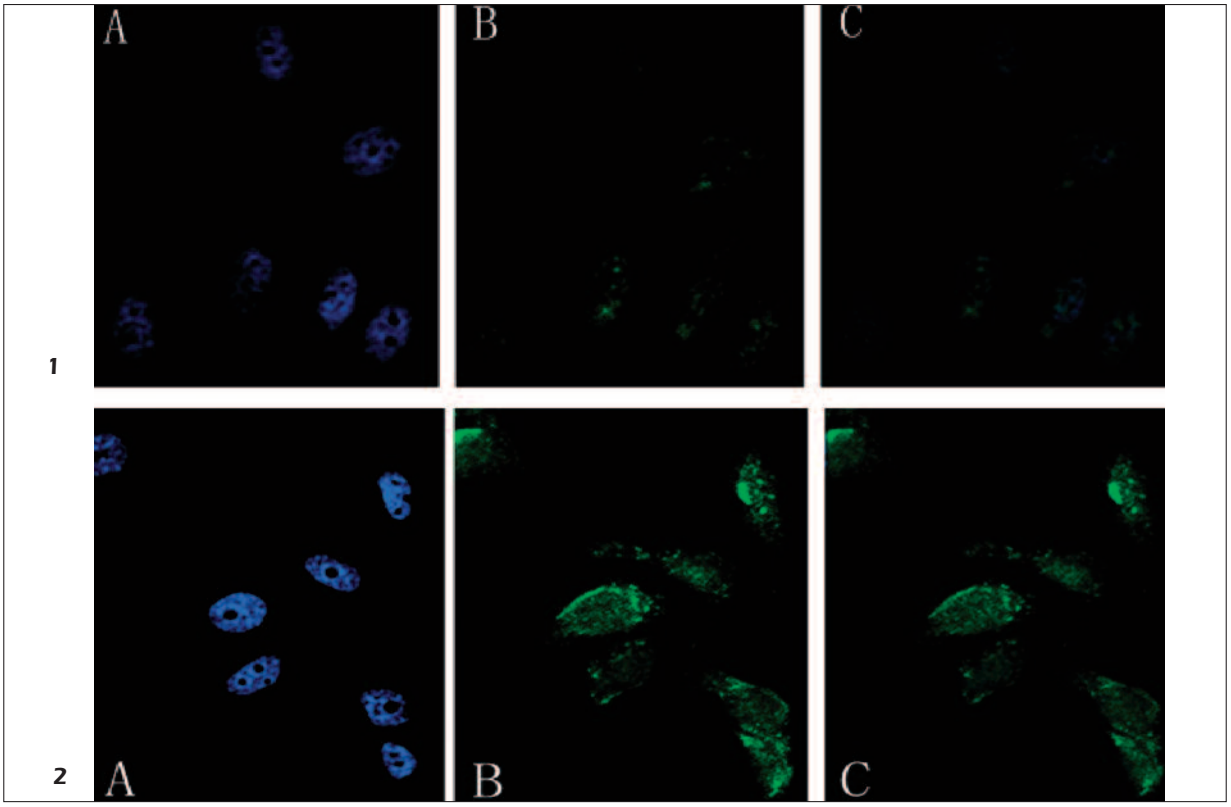

Figure 9. The distribution and expression of phospho-ERK1/2 in rabbit nucleus pulposus cell (×200). ***1.*** Nucleus pulposus cells cultured in 300 mOsm medium. ***2,*** Nucleus pulposus cells treated with 600 mOsm medium for 3h. **A,** Regions stained by DAPI revealing the nuclear structure of the cells. **B,** The expression of phospho-ERK1/2 in cytoplasm and nucleus. **C,** Confocal image of above images.

ly, such as p38 MAPK, JNK1/2, and ERK1/2; so these signal pathways seem to be involved in the observed hyperosmotic stress-induced nucleus pulposus cell apoptosis. Our findings may help to further elucidate the pathomechanism in conditions that involve increased osmotic pressure such as mechanical trauma and loads and make some MAPK inhibitors prevented intervertebral disc degeneration possible.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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