Lithium prevents glucocorticoid-induced chondrocyte autophagy: An *in vitro* study

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Abstract. Glucocorticoids can induce chondrocyte autophagy. Lithium is a classical regulator of autophagy. The present study aimed to determine whether lithium can prevent glucocorticoid-induced chondrocyte autophagy by regulating the PI3K/AKT/mTOR signaling pathway. For this purpose, rat and human chondrocytes were treated with dexamethasone (200 μM) or dexamethasone (200 μM) combined with lithium chloride at various concentrations (0.01, 0.1, 1 and 10 mM). CYTO-ID® autophagy fluorescence staining and transmission electron microscopy were used to detect the levels of autophagy in the chondrocytes. Reverse transcription-quantitative PCR and western blot analysis were used to measure the expression levels of the autophagy marker, LC3B and the autophagy regulatory signaling pathway (PI3K/AKT/mTOR signaling pathways) markers, AKT and mTOR. The viability of chondrocytes was measured using the Cell Counting Kit-8 assay. It was found that compared with that in the control group, dexamethasone induced the autophagy of chondrocytes, decreased the expression levels of AKT and mTOR, and reduced cell viability. Compared with the treatment with dexamethasone alone, lithium chloride (10 mM) + dexamethasone reduced the autophagy levels, increased the expression level of AKT and mTOR, and increased cell viability. In conclusion, the present study demonstrated that lithium can prevent glucocorticoid-induced autophagy by activating the PI3K/AKT/mTOR signaling pathway and preventing the glucocorticoid-induced decrease in chondrocyte viability.

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Introduction

Glucocorticoids (GCs) are drugs that are widely used in the treatment of local or systemic chronic inflammatory or autoimmune diseases (1,2), and are also frequently used in the enhanced recovery protocol following certain types of surgery such as total knee arthroplasty (3-5). However, the prolonged use of GCs sometimes results in side effects related to the skeletal system and cartilage (1,6). Previous studies reported that GCs can induce chondrocyte autophagy and the increased level of autophagy is associated with a reduction in cell viability (7-9). Abnormal levels of chondrocyte autophagy are closely related to the occurrence and development of certain osteoarticular diseases, such as Kashin-Beck disease and osteoarthritis (10,11).

Autophagy is a process through which eukaryotic lysosomes provide nutrients to cells by degrading damaged organelles, misfolded proteins and intracellular pathogens (12). The PI3K/AKT/mTOR signaling pathway is one of the classic autophagy regulatory signaling pathways, and it is currently the only known inhibitory pathway of autophagy (13). The activation of the PI3K/AKT/mTOR signaling pathway can inhibit autophagy, while the inhibition of this pathway induces autophagy (13).

Lithium is a classical regulator of autophagy (14). Previous studies reported that lithium can inhibit the apoptosis of bone marrow-derived mesenchymal stem cells (BMSCs) induced by serum deprivation or maintain the proliferative ability of liver cells by activating autophagy (15,16). Lithium also exerts neuroprotective effects by regulating the levels of autophagy in mouse models of hypoxic-ischemic encephalopathy and Alzheimer's disease (17,18). However, to the best of our knowledge, no studies available to date have examined the effects of lithium on chondrocyte autophagy. Thus, the present study aimed to determine whether lithium can prevent GC-induced chondrocyte autophagy by regulating the PI3K/AKT/mTOR signaling pathway.

Materials and methods

Ethics approval. The present study was approved to use commercially available primary cells by the Clinical Trials and Biomedical Ethics Committee of Sichuan University West China Hospital (approval no. 2021-102).

Cells and cell culture. All chondrocytes used in the present study were primary rat (cat. no. RAT-iCell-s003; each vial containing >5x10^5 cells in 1 ml volume) or human chondrocytes (cat. no. HUM-iCell-s018; each vial containing >5x10^5 cells in 1 ml volume), and were provided by iCell Bioscience Inc. The cells were cultured in a specialized primary chondrocyte culture system (cat. no. PriMed-iCell-020; iCell Bioscience Inc.).

CYTO-ID® autophagy fluorescence staining. The CYTO-ID Autophagy Detection kit (Enzo Life Sciences, Inc.) detects autophagy vesicles using novel dyes that selectively label the accumulation of autophagy vesicles so that they exhibit bright fluorescence. Primary rat and human chondrocytes at the logarithmic growth phase were prepared into a single-cell suspension. The cell density was adjusted to 1x10^5 cells/ml and 1 ml cell suspension was transferred in each well of 12-well plate. The cells were either left untreated (blank control), or treated with dexamethasone (200 μM; Beijing Solarbio Science & Technology Co., Ltd.), or dexamethasone (200 μM) combined with lithium chloride (MilliporeSigma) at various concentrations (0.01, 0.1, 1 or 10 mM), with four replicates in each group. The concentration of dexamethasone was determined according to a previous study (7) and our preliminary experiments. A previous study showed that 200 μM dexamethasone could increase the autophagy level of chondrocytes and significantly reduce cell viability (7). Similar results have been obtained from our preliminary experiments (unpublished data). After the cells adhered to the walls, each well was added with the drugs corresponding to the aforementioned grouping, followed by incubation for 24 or 48 h at 37˚C in an incubator with 5% CO_2. Subsequently, 300 μl pre-configured CYTO-ID staining solution was added to each well, and the cells were stained for 20 min at 37˚C in an incubator with 5% CO_2. The cells were then observed and photographed under an inverted fluorescence microscope (Zeiss AG). Under an x20 magnification field of view, five fields of view were randomly selected and the average fluorescence intensity was semi-quantitatively calculated using ImageJ software (version 1.8.0; US National Institutes of Health).

After screening for the suitable lithium chloride concentration (10 mM) and the duration of action (48 h) (under these conditions, the autophagy level of chondrocytes was changed most significantly), the following experiments were performed under these conditions.

Transmission electron microscopy (TEM). Primary chondrocytes at the logarithmic growth phase were prepared into a single-cell suspension. The cell density was adjusted to 1x10^5 cells/ml and 2 ml of cell suspension was transferred into each well of three 6-well plates. The cells were either left untreated, or treated with dexamethasone (200 μM), or dexamethasone (200 μM) combined with lithium chloride (10 mM) for 48 h at 37˚C in an incubator with 5% CO_2, with four replicates in each group. The cells were washed with PBS twice, digested and collected using trypsin. Subsequently, the cell suspensions were centrifuged at 250 x g for 5 min at room temperature, the supernatants were discarded and 0.5% glutaraldehyde fixation solution was slowly added along the tube wall using a dropper. Following resuspension, the cell suspension was placed in an environment at 4˚C for 10 min for fixation, and the cell suspension was then transferred into a 1.5-ml tip bottom EP tube and centrifuged at 12,000 rpm for 10 min at 4˚C. The supernatant was gently discarded, whereas the precipitate was retained and 3% glutaraldehyde fixing solution was slowly added using a dropper. The samples were then observed under a transmission electron microscope (JEM-1400FLASH, JEOL, Ltd.). A total of five normal cells were randomly selected from each sample and autophagosomes in the cytoplasm were counted. The average number of autophagosomes was compared between groups.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). The cell density was adjusted to 1x10^5 cells/ml and 2 ml of cell suspension was transferred into each well of three 6-well plates. The cells were either left untreated, or treated with dexamethasone (200 μM), or dexamethasone (200 μM) combined with lithium chloride (10 mM) for 48 h at 37˚C in an incubator with 5% CO_2, with four replicates in each group to extract RNA. To evaluate the expression levels of autophagy-related genes, including LC3B, AKT and mTOR, total RNA was extracted from the chondrocytes using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Total RNA was then reverse-transcribed into cDNA using the PrimeScript™ RT reagent kit (Promega Corporation). Reactions were performed using a 20 μl final volume with 2 μl cDNA and 10 μl SYBR™-Green PCR Master Mix (Promega Corporation). The nucleotide sequences of the PCR primers are presented in Table I.

Cycle threshold (Ct) values were obtained using Thermo Scientific PikoReal software (version 2.2; Thermo Fisher Scientific, Inc.). Relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method and normalized to the internal reference gene β-actin (19).

Cell protein extraction and western blot analysis. The cell density was adjusted to 1x10^5 cells/ml and 2 ml of cell suspension was transferred into each well of three 6-well plates. The cells were either left untreated, or treated with dexamethasone (200 μM), or dexamethasone (200 μM) combined with lithium chloride (10 mM) for 48 h at 37˚C in an incubator with 5% CO_2, with four replicates in each group to extract the protein. RIPA buffer (Wuhan Servicebio Technology Co., Ltd.) was used to lyse the rat and human chondrocytes from on ice for 10 min. The lysate mixture was then centrifuged at 12,000 rpm for 10 min at 4˚C. The BCA Protein Assay kit (Beyotime Institute of Biotechnology) was used to measure the total protein concentration and 20 μg protein/lane was separated by SDS-PAGE on a 10% or 12% gel. The separated proteins were subsequently transferred onto a PVDF membrane and blocked for 2 h at 25˚C in a PBS solution containing 5% skim milk powder. Subsequently, the membranes were incubated overnight at 4˚C with primary antibodies against LC3B (rabbit; 1:2,000; cat. no. ab192890; Abcam), phosphorylated (p-)AKT (rabbit; 1:1,000; cat. no. 310021; Chengdu Zen Bioscience Co., Ltd.), AKT (rabbit; 1:1,000; cat. no. 382804; Chengdu Zen Bioscience Co., Ltd.), phosphorylated mTOR (mouse; 1:1,000; cat. no. sc-293133; Santa Cruz Biotechnology, Inc.), mTOR (mouse; 1:1,000; cat. no. sc-517464; Santa Cruz Biotechnology, Inc.), and β-tubulin (rabbit; 1:2,000;

Table I. Nucleotide sequences of PCR primers.

	Primers for rat chondrocytes		Primers of human chondrocytes	
Gene IDs	Forward	Reverse	Forward	Reverse
ACTB	5'-CATCACTATCGGCA ATGAGCGGTTCC-3'	5'-ACGCAGCTCAGT AACAGTCCGCCTA-3'	5'-CATGTACGTTG CTATCCAGGC-3'	5'-CTCCTTAATGT CACGCACGAT-3'
AKT	5'-AACGGCAGGAGGA GGAGACGATGGA-3'	5'-CTCGTTCATGGTC ACACGGTGCTTGG-3'	5'-TGACCATGAAC GAGTTTGAGTA-3'	5'-GAGGATCTTCA TGGCGTAGTAG-3'
LC3B	5'-CCGTCCTGGACAA GACCAAGTTCCT-3'	5'-ACACTCACCATGC TGTGCCCATTCA-3'	5'-GCCGTCGGAGA AGACCTTCAAG-3'	5'-TGGTTGGATGC TCTCGAATAAG-3'
m-TOR	5'-AGAGGACCAGCAG CACAAGCAGGAG-3'	5'-GCAGTGGTGGTGG CATTGGTGATGTT-3'	5'-GAGATACGCTG TCATCCCTTTA-3'	5'-CTGTATTATTGA CGGCATGCTC-3'

ACTB, β-actin.

cat. no. AF7011; Affinity Biosciences). After washing with PBST, the membranes were incubated with the secondary polyclonal goat anti-rabbit/mouse HRP-conjugated antibodies (cat. no. ZDR-5306 and ZDR-5307; both 1:5,000; OriGene Technologies, Inc.) at room temperature for 2 h. Finally, after washing the membranes with PBST, the protein bands were visualized using the ECL method (Torchlight Hypersensitive ECL Western HRP Substrate; cat. no. 17046; Chengdu Zen Bioscience Co., Ltd.) and the Quantity One software (version 4.6.6; Bio-Rad Laboratories, Inc.) was used to analyze the results with β-tubulin as the loading control.

Cell Counting Kit-8 (CCK-8) assay. To measure the cell viability, the cell density was adjusted to $3x10^4$ cells/ml and 100 μl of cell suspension was transferred into each well of a 96-well plate. The cells were either left untreated, or treated with dexamethasone (200 μM), or dexamethasone (200 μM) combined with lithium chloride (10 mM) for 48 h at 37˚C in an incubator with 5% CO_2, with four replicates in each group. The CCK-8 reagent (Apexbio Technology LLC) was diluted 1:10 and 110 μl diluted CCK-8 solution was added into each well. After 2 h, the optical density (OD) value of each well was examined at 450 nm according to the manufacturer's instructions. Cell viability was calculated as follows: Cell viability (%)=(OD value of observation group-OD value of zero adjustment group)/(OD value of control group-OD value of zero adjustment group) x100%.

Statistical analysis. Statistical analysis was performed using SPSS 26.0 software (IBM Corp.). For continuous data, one-way analysis of variance (ANOVA) was used and Tukey's test was used as the post hoc test. Continuous data are presented as the mean ± standard deviation unless otherwise indicated. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of GC and lithium on the chondrocyte autophagy level. The results of CYTO-ID autophagy fluorescence staining revealed that the autophagy level of rat chondrocytes increased significantly following treatment with dexamethasone for 24 and 48 h (Fig. 1). Following treatment with dexamethasone combined with various concentrations of lithium chloride for 24 and 48 h, it was found that 10 mM lithium chloride for 48 h significantly reduced the autophagy level overactivated by dexamethasone in rat chondrocytes.

The results of CYTO-ID autophagy fluorescence staining revealed that the autophagy level of human chondrocytes did not change significantly following treatment with dexamethasone for 24 h and the autophagy level of human chondrocytes increased significantly following treatment with dexamethasone for 48 h (Fig. 2). Following treatment with dexamethasone combined with various concentrations of lithium chloride for 24 and 48 h, it was found that 10 mM lithium chloride for 48 h significantly reduced the autophagy level overactivated by dexamethasone in human chondrocytes. Therefore, the rat and human chondrocytes were divided into three groups in subsequent experiments: The control group was composed of cells untreated; the dexamethasone group was composed of cells treated with 200 μM dexamethasone for 48 h; the lithium group was composed of cells treated with 200 μM dexamethasone and 10 mM lithium chloride for 48 h.

Compared with the control and lithium groups, a significantly greater number of autophagosomes was observed by TEM in the dexamethasone group of rat chondrocytes (Fig. 3A and C). Compared with the control and lithium groups, a significantly greater number of autophagosomes was observed by TEM in the dexamethasone group of human chondrocytes (Fig. 3B and D).

Effects of GC and lithium on cell viability. The results of the CCK-8 assay of rat chondrocytes revealed that the average cell viability of the dexamethasone group was significantly lower than that of the control and lithium groups, and the average cell viability of the lithium group was significantly lower than that of the control group (Fig. 4A). The results of the CCK-8 assay of human chondrocytes revealed that the average cell viability of the dexamethasone group was significantly lower than that of the control and lithium groups, and the average cell viability of the lithium group was significantly lower than that of the control group (Fig. 4B).

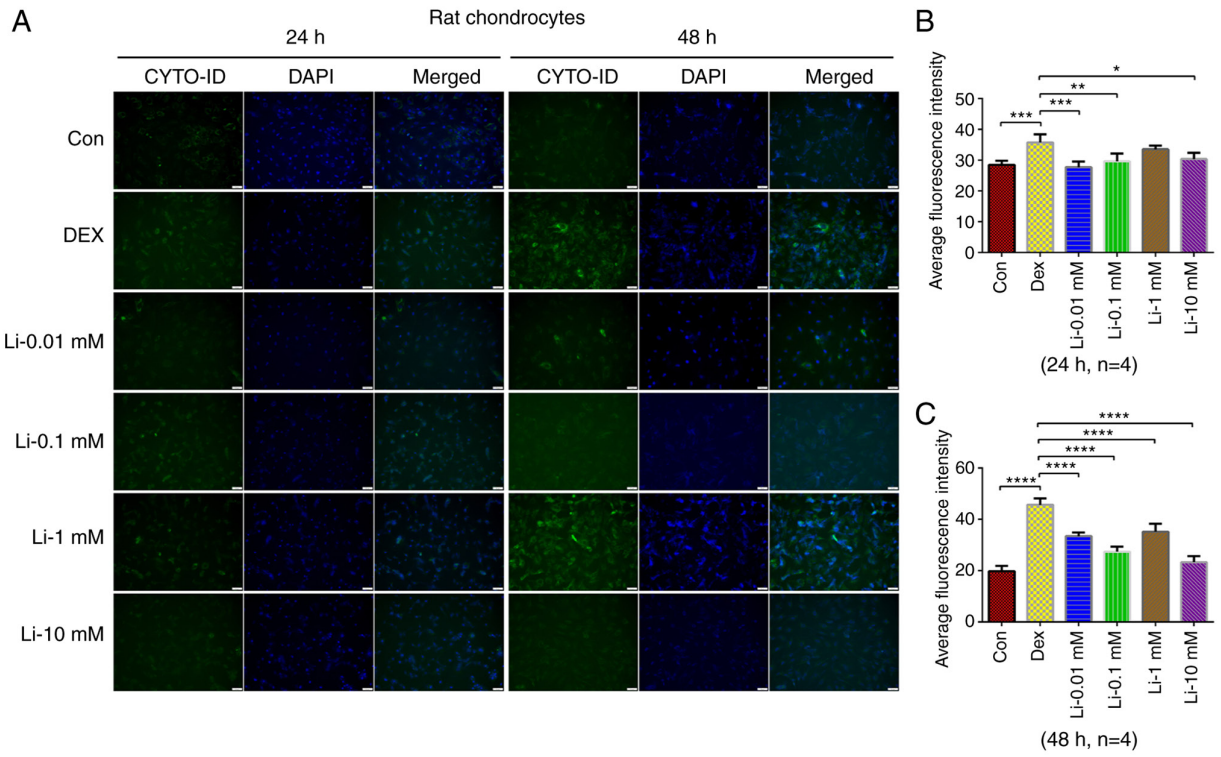

Figure 1. CYTO-ID autophagy fluorescence staining of rat chondrocytes either left untreated (control group), or treated with dexamethasone (200 μM) and dexamethasone (200 μM) combined with various concentrations of lithium chloride (0.01, 0.1, 1 or 10 mM). (A) Representative images of CYTO-ID autophagy fluorescence staining (magnification, x200). Average fluorescence intensity of CYTO-ID autophagy fluorescence staining at (B) 24 and (C) 48 h. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Con, control group; DEX, dexamethasone group; Li, dexamethasone combined with lithium chloride group.

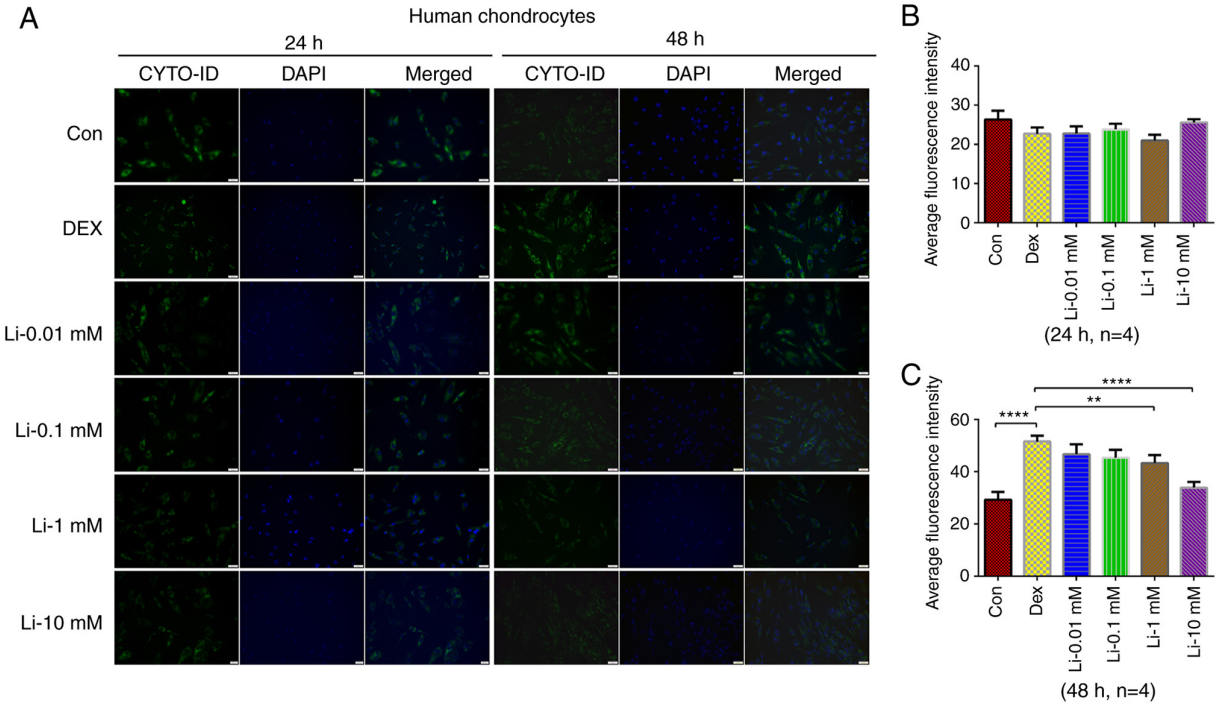

Figure 2. CYTO-ID autophagy fluorescence staining of human chondrocytes either left untreated (control group), or treated with dexamethasone (200 μM) and dexamethasone (200 μM) combined with various concentrations of lithium chloride (0.01, 0.1, 1 or 10 mM). (A) Representative images of CYTO-ID autophagy fluorescence staining (magnification, x200). Average fluorescence intensity of CYTO-ID autophagy fluorescence staining at (B) 24 and (C) 48 h. **P<0.01 and ****P<0.0001 Con, control group; DEX, dexamethasone group; Li, dexamethasone combined with lithium chloride group.

Expression of genes related to autophagy in chondrocytes. The results of RT-qPCR of rat chondrocytes revealed that the relative expression of LC3B in the dexamethasone group was significantly higher than that of the control and lithium

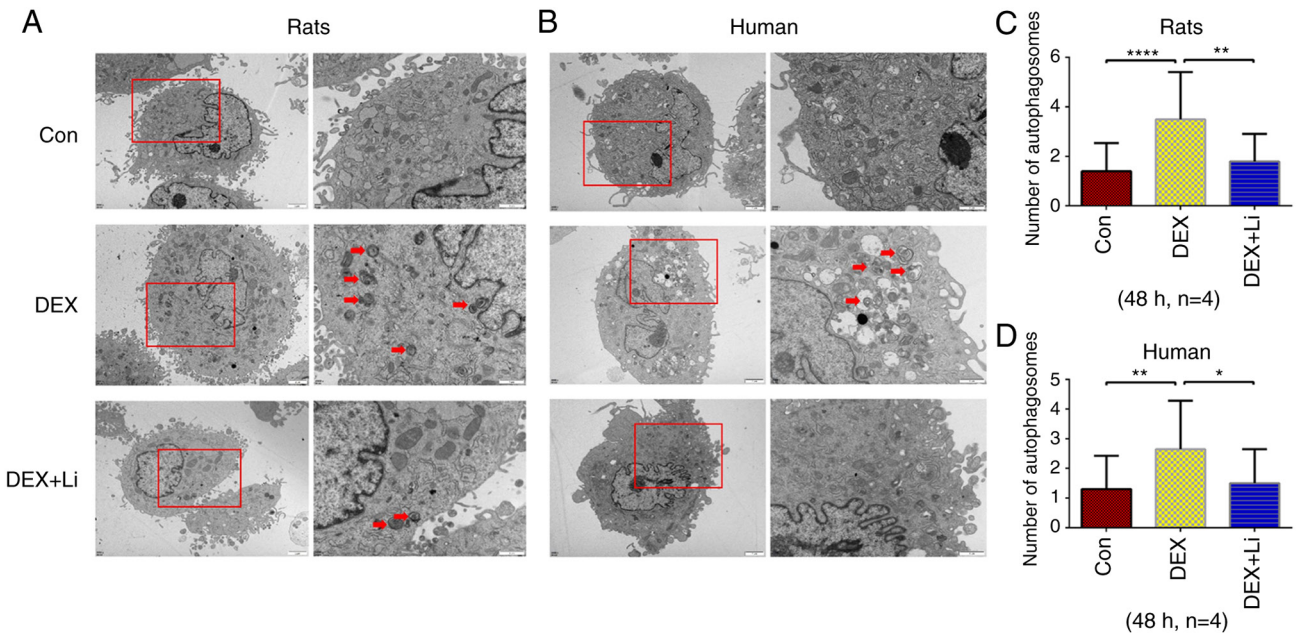

Figure 3. Results of transmission electron microscopy. Representative images of (A) Rat and (B) Human chondrocytes. The boxes in the images on the left panel (magnification, x6,000) indicate the areas enlarged on the images on the right panel (magnification, x24,000). Red arrows indicate autophagosomes. The average number of autophagosomes from (C) Rat and (D) Human chondrocytes. *P<0.05, **P<0.01 and ****P<0.0001. Con, control group; DEX, dexamethasone group (200 μM); DEX + Li, dexamethasone (200 μM) combined with lithium chloride (10 mM) group.

groups, while the relative expression of AKT and mTOR in the dexamethasone group was significantly lower than that of the control and lithium groups (Fig. 4C-E). The results of RT-qPCR of human chondrocytes revealed that the relative expression of LC3B in the dexamethasone group was significantly higher than that of the control and lithium groups, while the relative expression of AKT and mTOR in the dexamethasone group was significantly lower than that of the control and lithium groups (Fig. 4F-H).

The results of western blot analysis of rat chondrocytes revealed that the relative expression of LC3II/I in the dexamethasone group was significantly higher than that of the control and lithium groups, while the relative expression of phosphorylated AKT/AKT and phosphorylated mTOR/mTOR in the dexamethasone group was significantly lower than that of the control and lithium groups (Fig. 5A-D). The results of western blot analysis of human chondrocytes revealed that the relative expression of LC3II/I in the dexamethasone group was significantly higher than that of the control and lithium groups, while the relative expression of phosphorylated AKT/AKT and phosphorylated mTOR/mTOR in the dexamethasone group was significantly lower than that of the control and lithium groups (Fig. 5E-H).

Discussion

To the best of our knowledge, the present study is the first to identify that lithium can prevent GC-induced autophagy by activating the PI3K/AKT/mTOR signaling pathway, as well as prevent the GC-induced decrease in the viability of chondrocytes.

GCs can affect the normal physiological function of chondrocytes in some diseases of the skeletal system, such as osteoarthritis and skeletal dysplasia (6,20-24). For example, GCs can promote chondrocyte apoptosis and inhibit chondrocyte viability (22,23). Apoptosis may reduce the number of chondrocytes and decrease the extracellular matrix components, including proteoglycan and collagen type II, contributing to osteoarthritis (24). In addition, the decreased proliferation of chondrocytes has been linked to the thinning of the growth plate, thus leading to an impairment in skeletal development and growth (2,25).

It was previously found that GCs can induce chondrocyte autophagy and this is associated with a reduction in cell viability (7-9). A recent study reported that GC-induced osteonecrosis of the femoral head was associated with abnormal chondrocyte hyperplasia in articular surface cartilage, which may be related to the GC-induced overactivation of autophagy in chondrocytes (26). The change in the level of autophagy of chondrocytes is also related to the pathogenesis of osteoarthritis (27-29). At present, it is generally considered that autophagy, as an adaptive response, can reduce chondrocyte death in the early stages of osteoarthritis; however, with the development of osteoarthritis, excessive autophagy may also cause chondrocyte death (27); therefore, GC-induced chondrocyte autophagy may cause certain types of pathological changes.

Lithium, a common drug used in the treatment of psychosis, is also an autophagy regulator (14). Recently, lithium has been found to regulate autophagy levels in cells of the skeletal system (30). The same study reported that lithium chloride reversed the effects of ovariectomy on BMSCs extracted from ovariectomized mice, promoting osteogenesis and suppressing apoptosis by regulating autophagy (30). In addition, lithium chloride was found to regulate autophagy, decrease apoptosis and promote bone formation, thus protecting tooth movement in osteoporotic mice (30). In the present study, it was first found that lithium chloride maintained the viability of

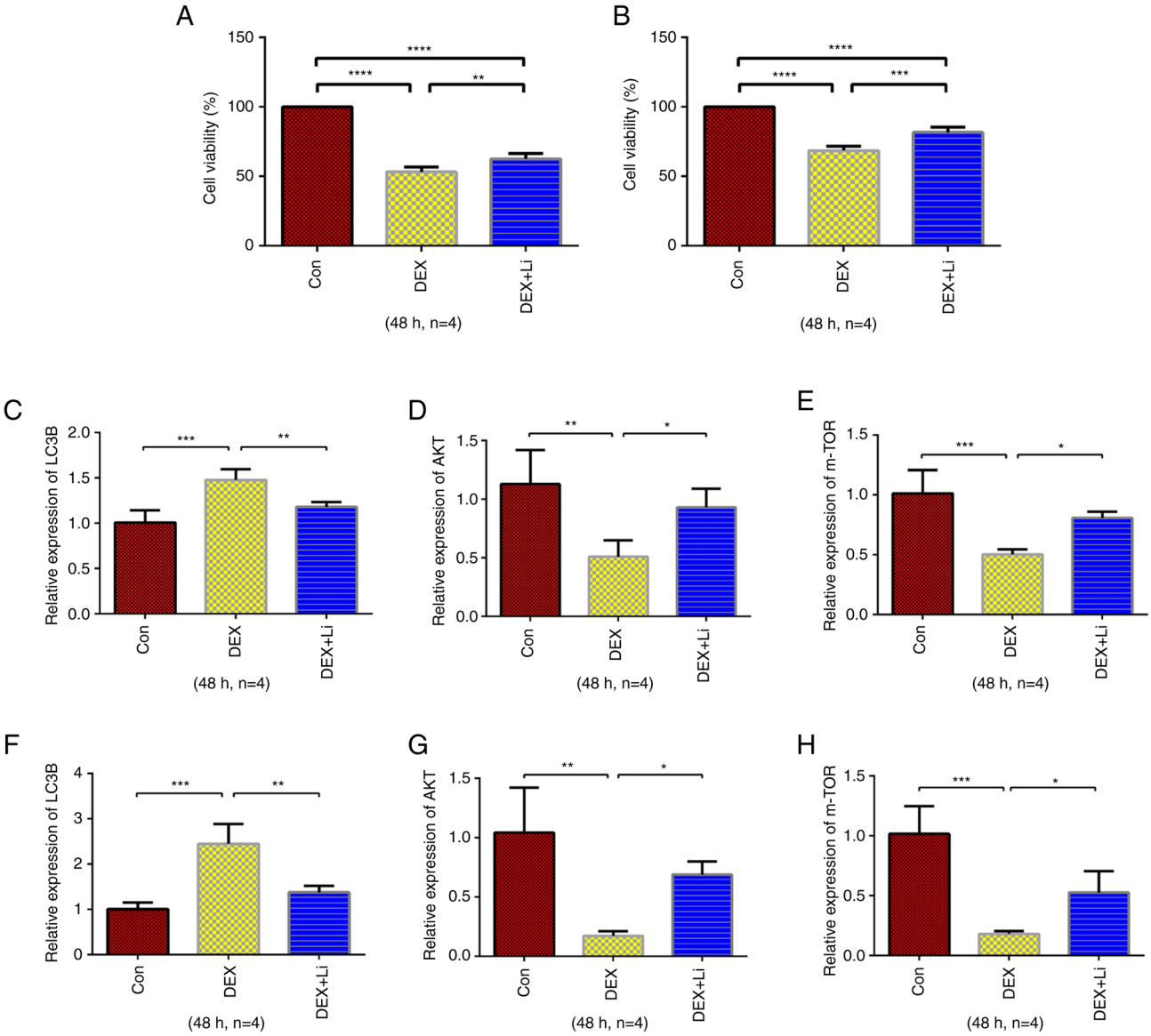

Figure 4. Results of CCK-8 assay and RT-qPCR. CCK-8 assay of (A) rat and (B) human chondrocytes. Relative mRNA levels of (C) LC3B, (D) AKT and (E) mTOR from rat chondrocytes. Relative mRNA levels of (F) LC3B, (G) AKT and (H) mTOR from human chondrocytes. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Con, control group; DEX, dexamethasone group (200 μM); DEX + Li, dexamethasone (200 μM) combined with lithium chloride (10 mM) group; CCK-8, Cell Counting Kit-8; RT-qPCR, reverse transcription-quantitative PCR.

chondrocytes by regulating autophagy. Lithium may thus have immense potential for use in the field of diseases related to abnormal chondrocyte autophagy levels, such as osteoarthritis and Kashin-Beck disease (10,11,27-29). A previous study indicated that lithium-containing scaffolds are effective in promoting cartilage regeneration (31). However, the underlying mechanisms of cartilage regeneration mediated by lithium-containing biomaterials remain unclear.

A recent study reported that the treatment of BMSC-derived exosomes (Li-BGC-Exo) with lithium chloride markedly facilitated cartilage regeneration *in vivo* (32). In that previous study, the researchers selected a lithium-substituted bioglass ceramic (Li-BGC) model and systematically evaluated the regulatory role of Li-BGC-Exo following Li-BGC treatment. The results revealed that Li-BGC-Exo significantly promoted chondrogenesis, which was attributed to the upregulation of exosomal miR-455-3p transfer, resulting in the inhibition of histone deacetylase 2 and the enhanced acetylation of histone H3 in chondrocytes (32). These findings suggest that lithium may promote cartilage repair through other mechanisms in addition to the regulation of autophagy.

Several signaling pathways were reported to be associated with the level of autophagy in cells and several key molecules can regulate the autophagy pathway (27). The PI3K/AKT/mTOR signaling pathway is one of the classic autophagy regulatory signaling pathways and this pathway is currently the only known inhibitory pathway of autophagy (13). A previous study explored the association between the levels of autophagy of articular chondrocytes in rats with osteoarthritis and the PI3K/AKT/mTOR signaling pathway (28). That previous study found that inflammation inhibited the proliferation of rat chondrocytes and reduced the rate of autophagy. The inhibition of the PI3K/AKT/mTOR signaling pathway promoted chondrocyte autophagy and reduced inflammation (28).

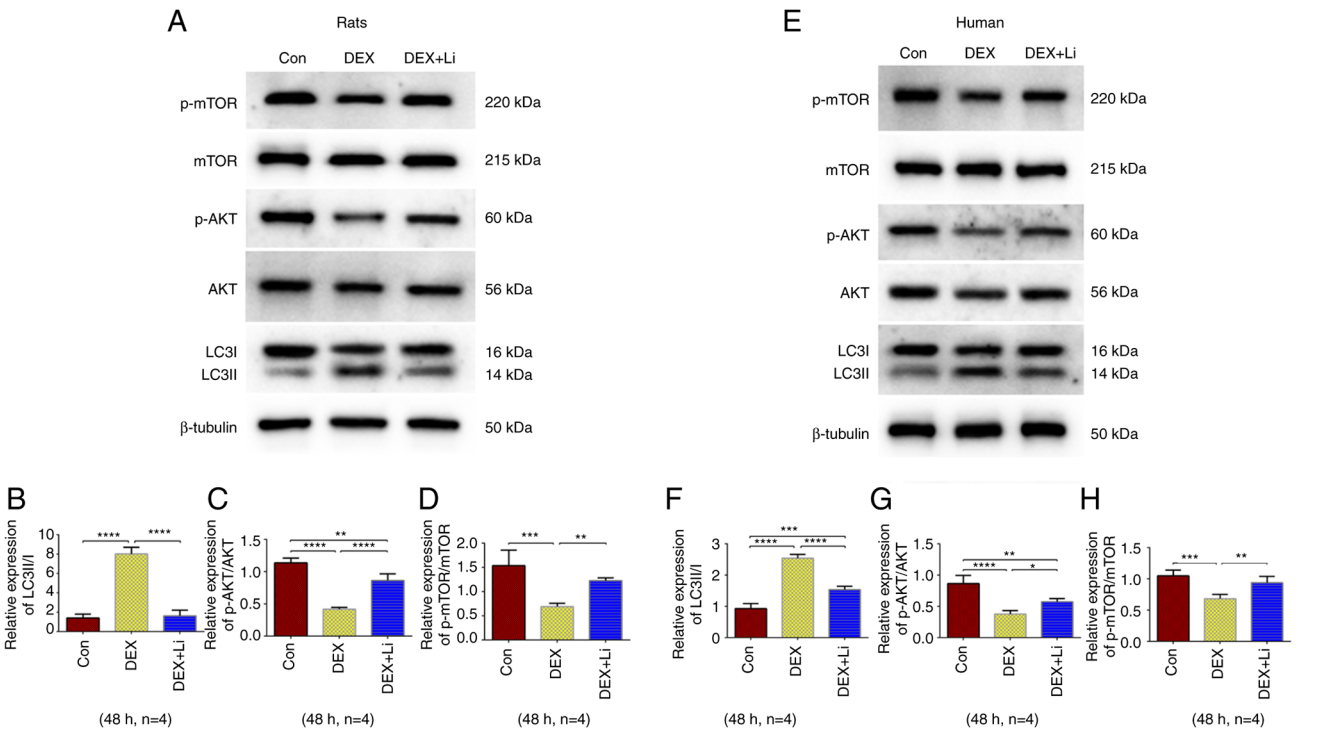

Figure 5. Results of western blot analysis. (A) Representative western blots of rat chondrocytes. The average relative expression of (B) LC3II/I, (C) p-AKT/AKT and (D) p-mTOR/mTOR from rat chondrocytes. (E) Representative western blots of human chondrocytes. The average relative expression of (F) LC3II/I, (G) p-AKT/AKT, and (H) p-mTOR/mTOR from human chondrocytes. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Con, control group; DEX, dexamethasone group (200 μM); DEX + Li, dexamethasone (200 μM) combined with lithium chloride (10 mM) group; p-AKT, phosphorylated AKT; p-mTOR, phosphorylated mTOR; p-, phosphorylated.

The findings of the present study suggested that lithium could also prevent GC-induced autophagy by activating the classic PI3K/AKT/mTOR signaling pathway. However, the PI3K/AKT/mTOR signaling pathway is a complex signaling pathway with multiple regulators and effectors. Most importantly, this signaling pathway is essential for the normal physiological function of chondrocytes. It was previously suggested that targeting this pathway may be a viable treatment option for osteoarthritis (33). However, merely activating or inhibiting the PI3K/AKT/mTOR signaling pathway to prevent or treat osteoarthritis can be a double-edged sword, as the side effects of this approach appear inevitable (33). For example, the PI3K/AKT/mTOR signaling-mediated synovial inflammation, subchondral bone sclerosis, extracellular matrix homeostasis, chondrocyte proliferation, apoptosis, autophagy and inflammation greatly affect cell fate and OA pathophysiology. There will be an imbalance among these cell processes if simply activating or inhibiting PI3K/AKT/mTOR signaling. Therefore, future studies are required to further elucidate the role of the PI3K/AKT/mTOR signaling pathway in the different pathophysiological stages of osteoarthritis and clarify the specific molecular mechanisms, such as how this signaling pathway interacts with other signaling pathways. In addition, it needs to be determined how to target this pathway in osteoarthritis without affecting the regulatory processes of other critical physiological signaling pathways. If these issues are addressed, PI3K/AKT/mTOR-based osteoarthritis treatments may become effective, safe and promising.

Previous studies reported that the induction of autophagy by dexamethasone can be inhibited by 3-methyladenine, an autophagy inhibitor, and RU486, a GC antagonist, and the inhibitory effects are concentration-dependent in a certain range (100-400 μM for 3-methyladenine, and 10-300 μM for RU486) (7,8). In the present study, it was found that treatment with 10 mM lithium chloride for 48 h significantly reduced the autophagy level overactivated by dexamethasone. Lithium chloride functioned as an autophagy regulator and played a role by activating the PI3K/AKT/mTOR signaling pathway. Herein, autophagy inhibitors were not used to compare the ability of lithium to inhibit autophagy directly.

Previous studies on chondrocyte autophagy mostly used rat and mouse chondrocytes (7-9,28). In the present study, both rat and human chondrocytes were used, and consistent results were obtained. Species-dependent effects were not observed.

The present study has two limitations. First, the results from cell studies were not verified or extended on animal models. It is important to further apply these findings and results using animal models, as it is well known that results can vary between *in vivo* and *in vitro* experiments. Second, autophagy inhibitors were not used to compare the ability of lithium to inhibit autophagy. Further studies are thus required to address these limitations.

In conclusion, under the conditions of the present study, lithium was shown to prevent GC-induced autophagy by activating the PI3K/AKT/mTOR signaling pathway, and it was also found to prevent the GC-induced decrease in chondrocyte viability. Lithium may thus have immense potential for use in the field of diseases related to abnormal chondrocyte autophagy levels, such as osteoarthritis and Kashin-Beck disease.

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Availability of data and materials

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

Authors' contributions

QW and WZ conducted the experiments and wrote the manuscript. JH, CZ and LC analyzed the data and assisted in the writing of the manuscript. PK oversaw the study and made important intellectual contributions to the manuscript. PK was involved in the conception and design of the study. QW and PK confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript. The authors clarify that no artificial intelligence (AI) tools were used in this study or when writing this manuscript.

Ethics approval and consent to participate

This study was approved to use commercially available primary cells by the Clinical Trials and Biomedical Ethics Committee of Sichuan University West China Hospital (approval no. 2021-102).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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