Polydatin Protects Against Articular Cartilage Degeneration by Regulating Autophagy Mediated by AMPK/Mtor Signaling Pathway

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Abstract

Background: Knee osteoarthritis (KOA) is one of the leading causes of disability, and its etiopathogenesis is not completely understood. Polydatin has the potential effect on the treatment of KOA, but the mechanism is not clear.

Methods: After an KOA rat model was established by anterior cruciate ligament transection, KOA rats were treated with polydatin (4 mg/kg) for 30 days. Subsequently, cartilage tissues were collected from rats and detected by HE, TUNEL staining and Western blotting to evaluate the pathological damage, apoptosis and autophagy activity. Then, human chondrocyte C28/I2 cells were stimulated by LPS to induce a KOA model in vitro, and the effects of polydatin on the C28/I2 cell viability, apoptosis and autophagy were also detected. In addition, the mechanism of polydatin on KOA in C28/I2 cells was investigated, and the effect of an AMPK inhibitor (Dorsomorphin 2HCl) on the proliferation and apoptosis of polydatin administrated-cells were also detected.

Results: After treated with polydatin, the pathological damage of rat cartilage tissues were ameliorated, cells apoptosis was inhibited and autophagy was activated in KOA rats. Meanwhile, polydatin also ameliorated the proliferation and apoptosis of C28/I2 cells, the expression of autophagy-related proteins, LC3II/LC3I, Beclin-1, and p-AMPK/AMPK were up-regulated, p-mTOR/mTOR was down-regulated by polydatin in C28/I2 cells. Interestingly, relative results showed that the improvement effect of polydatin on LPS-stimulated-C28/I2 cells was blocked by AMPK/mTOR inhibitor, Dorsomorphin 2HCl.

Conclusion: Our research showed that polydatin reduces apoptosis and activate autophagy both in a rat model of KOA and C28/I2 cell model by AMPK/mTOR signaling pathway, which provides the basis for further investigations into the potential therapeutic impact of polydatin in KOA.

Introduction

Knee osteoarthritis (KOA) is a multifactorial disease characterized by decreased muscle strength, limited function and narrowed joint spaces or even disability caused by severe pain [1]. It is a degenerative joint disease and often related to age, and the prevalence is increased followed the increased of age [2]. As the global population aging, KOA is becoming an increasing burden on society. At present, the therapeutic methods for KOA are limited and the most common treatments were analgesics and physical therapy. Unfortunately, joint replacement or osteotomy may be inevitable [3]. Therefore, it is urgent for researchers and practitioners to search a new prevention and treatment of KOA.

Owing to the complexity of KOA pathology, the main causes leading to KOA are still unclear [4]. The key factors of the degeneration of articular cartilage are considered as the extracellular matrix lost and chondrocytes died in KOA [5]. Usually, cells could adapt to the pressures of environmental by regulate autophagy and apoptotic. The apoptosis of extracellular matrix which synthesized and secreted from articular chondrocytes was associated with the pathogenesis of KOA [6]. At the same time, chondrocyte

autophagy also acts a key role in the process of KOA [7]. Therefore, the treatment which associated with the dysregulating apoptosis and autophagy of chondrocyte is useful.

Polydatin is isolated from the plant *Polygonum cuspidatum*, and is a kind of glycosylated polyphenol with a monocrystalline structure. It has a lot of positive health functions such as immunoregulatory, anti-inflammatory, anti-oxidative and anti-tumoral proprieties [8, 9]. In addition, polydatin can regulate the autophagy mediated by AMPK/mTOR signaling pathway. It can inhibit the proteins expression of PI3K/Akt/mTOR pathway to improve autophagic dysfunction and reduce atherosclerotic lesions [10]. And it also suppressed autophagy to protect against fructose-induced podocyte injury by its anti-oxidation activity [11].

Therefore, we established a KOA model both *in vivo* and *in vitro* to investigate the possibility effect of polydatin for the treatment of KOA. Then the regulation of polydatin on apoptosis and autophagy was investigated, and the potential mechanism was further explored.

Materials And Methods

In vivo

Animals

Thirty healthy adult Sprague-Dawley (SD) rats (250~280 g) were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd. (SCXK (Hu) 2017-0005, Shanghai, China). All the rats were allowed to access food and water free and living in standard condition (humidity: 55~70%; room temperature: 23±2 °C; light cycle: 12-h light/dark). All animal assays were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animal experiments were in accordance with the guidelines of laboratory animal care and were approved by the Hangzhou Eyong Biotechnological Co., Ltd. Animal Experiment Center (Hangzhou, China).

Established KOA model and experimental group

The rats were divided into 3 groups randomly (n=10/group): control, KOA and KOA + polydatin. The KOA was induced according to previously study [12]. In detail, after anesthetized with pentobarbital (40 mg/kg), the KOA and KOA + polydatin rats were fixed in supine position. And the right medial knee joint was made a longitudinal incision (1.5 cm), the medial collateral ligament and joint capsule was exposed and broken. Then dislocated the patella to expose and cut off the anterior cruciate ligament. Later, the medial meniscus was extirpated and cut off the posterior crucial ligament. Finally, established KOA rat models were verified by drawer test. After fixed the patella, sutured the joint capsule and skin. In order to avoid infection, all the rats were treated with penicillin (40,000 U/mL, 1 mL) by intramuscularly injected after the surgery for consecutive 3 days. The normal group rats not received any treatment. All the rats were fed and drank freely and normally. After modeling 4 weeks [13], the KOA + polydatin rats were

intraperitoneally injected with polydatin (4 mg/kg, dissolved in saline), while normal rats and model rats intraperitoneally injected with same volume normal saline and continued for 30 d.

Hematoxylin-eosin (HE) staining

The cartilage tissues of each group rats were fixed in 4% paraformaldehyde for 24 h at room temperature. Then, the cartilage tissues were routinely dehydrated by gradient ethanol, embedded, and cut into 4 μ m sections. Then the sections were stained with HE. After washed, the slices were observed under an optical microscope (Olympus, Japan) to detected the histopathological changes. The cartilage tissues were scored as following: 0 point, normal; 1 point, mild cell infiltration and synovial invasion; 2 points, bone invasion; 3 points, more serious cell infiltration and synovial invasion accompanied by bone invasion; 4 points, severe cell infiltration and synovial invasion accompanied by bone invasion.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

The apoptosis rate of chondrocytes was assessed by TUNEL assay. After fixed with 4% paraformaldehyde and dehydrated, the brain tissue was embedded and sectioned into 4- μ m. When the slices were dewaxed and transparent, 100 μ L TdT enzyme buffer was added and incubated at 37 °C for 1 h in a dark and humectation environment. Then the slices were incubated with 100 μ L Streptavidin-HRP at 37 °C for 30 min. After washed, the sections were stained with diaminobenzidine (DAB) (MD912068, MDL, China), and re-stained with hematoxylin. Six fields were randomly selected for each group and quantitative analysis was detected by Image J. Apoptosis index (%) = TUNEL-positive neurons/total neurons $\times$ 100%.

Western blotting

Radioimmunoprecipitation assay (RIPA) was used to extracted protein from the ischemic cartilage tissues. After crushed in liquid nitrogen, the tissue lysates were collected and centrifugated at 12000 g for 20 min to obtained the supernatant. After quantified the concentration of total cellular proteins by bicinchoninic acid (BCA, pc0020, Solarbio, China) kit. The proteins were loaded onto SDS-PAGE gel, separated by electrophoresis, and transferred onto a polyvinylidene difluoride (PVDF) membrane (GE Healthcare Life, USA). After blocked by 5% dried skim milk for 30 min, primary antibodies were added and incubated with membranes overnight at 4 °C, then incubated with secondary antibody at room temperature for 1 h. Enhanced chemiluminescence (ECL) (Solarbio, Beijing, China) method was used to detected the expression of protein. Image J software (National Institutes of Health) was used to detected the band intensities. The relative protein level was normalized to that of GAPDH. The primary antibodies including anti-LC3A/B antibody (1:500, AF5402, Affinity, USA), anti-Beclin 1 antibody (1:500, AF5128, Affinity, USA), anti-GAPDH antibody (1:3000, AF7021, Affinity, USA).

In vitro

Cell culture and grouping

C28/I2 cell lines were cultured in DMEM/F12 medium (SH30243.01, Hyclone, USA) at 37 °C and under 5% CO₂ atmosphere. The medium was changed every 48 h. When the cells reached about 80-90% confluence, cells were used for the further experiments. The C28/I2 cell lines were seeded into six plates or flasks and divided into 3 groups: control (only DMEM/F-12), LPS (DMEM/F-12 contain 5 µg/mL LPS, cultured for 24 h), LPS+polydatin (DMEM/F-12 contain 5 µg/mL LPS and 1 µM polydatin, cultured for 24 h) [14].

MTT

In order to detected the effect of LPS and polydatin on C28/I2 cells, MTT assay was performed. Briefly, C28/I2 cells (1×10^5 cell/L, 100 µl/well) which was logarithmic growth phase were seeded into 96-well plates and incubated in a incubator (37°C, 5% CO₂) for 24 h. After treated with LPS and polydatin for 24 h, 10 µL MTT was added and cultured for another 24 h. Finally, the plates were detected at 450 nm.

Flow Cytometry

Logarithmic growth phase C28/I2 cells (1.2×10^6 /well) were seeded into 96-well plates. After induced by LPS and treated with polydatin for 24 h, the concentration of C28/I2 cells were diluted into 1×10^6 cells/mL by cool PBS. Then, annexin V/PI staining was processed to assess the anti-apoptotic effects of polydatin on C28/I2 cells induced by LPS. C28/I2 cells were re-suspend in binding buffer (100 µL), and co-incubated with Annexin V-FITC (5 µL) and PI solution (10 µL) at room temperature in the dark for 15 min. After added 400 µL binding buffer, the solution was analyzed by flow cytometry within 1 h.

Western blotting

After treated with LPS and polydatin, the C28/I2 cells were lysed with RIPA buffer to obtained protein. After measured the concentrations by BCA Kit, proteins were also separated by SDS-PAGE and transferred onto PVDF membranes. After blocked with 5% dried skim milk for 2 h, the membranes were incubated with dilute solution of antibodies at 4 °C overnight and secondary antibody for 2 h at room temperature. ECL method was used to detected the expression of protein. Image J software was used to detected the band intensities. The relative protein level was normalized to that of GAPDH. The primary antibodies including anti-LC3A/B antibody (1:500, AF5402, Affinity, USA), anti-Beclin 1 antibody (1:500, AF5128, Affinity, USA), phospho-AMPK alpha (Thr172) antibody (1:500, AF3423, Affinity, USA), anti-AMPK alpha antibody (1:500, AF6423, Affinity, USA), phospho-mTOR (Ser2448) antibody (1:500, AF3308, Affinity, USA) and anti-mTOR antibody (1:500, AF6308, Affinity, USA), anti-GAPDH antibody (1:3000, AF7021, Affinity, USA).

Effect of Dorsomorphin 2HCl on C28/I2 cells

The inhibitor of AMPK was used to assess the mechanism of polydatin on the C28/I2 cells. The C28/I2 cells were divided into four groups: control (only DMEM/F-12), LPS (DMEM/F-12 contain 5 µg/mL LPS, incubate for 24 h), LPS+polydatin (DMEM/F-12 contain 5 µg/mL LPS and 1 µM polydatin, incubate for 24

h), and LPS+polydatin+Dorsomorphin 2HCl (After treated with 10 μ M Dorsomorphin 2HCl for 1 h, C28/I2 cells were cultured in DMEM/F-12 with 5 μ g/mL LPS and 1 μ M polydatin for 24 h) [15]. Then, the role of Dorsomorphin 2HCl on the proliferation and apoptosis of C28/I2 cells and the mechanism were also detected according to the above description.

Statistical analysis

All the assay data were analyzed by SPSS 19.0 statistical software. The data were shown as $\bar{x} \pm s$. Multi-group comparison were analysed by One-way-ANOVA. Inter-group comparisons were analysed by SNK. Variance data were analysed by Kruskal-Wallis H test. $P < 0.05$ was seen as statistically significant.

Result

In vivo

Polydatin attenuate pathological changes of cartilage tissues in KOA rats

HE staining was performed to detected the pathological changes in of rats. Figure 1 reflected that the surface of knee cartilage tissues in control rats was smooth, the structure of cartilage was normal, chondrocytes were arranged orderly and no fracture in normal rats. However, compared with the control rats, the surface of cartilage was rough, chondrocytes were disordered, fracture, necrotic chondrocytes were observed in KOA rats ($P < 0.01$). Compared with the KOA rats, after treated with polydatin, there was less necrotic chondrocytes were observed, the arranged of chondrocytes and structure of cartilage was similar to control rats ($P < 0.01$).

Polydatin inhibit apoptosis of chondrocytes in KOA rats

TUNEL staining showed the apoptosis of chondrocytes in KOA rats (Figure 2). The TUNEL positive cell rate was significantly increased in KOA rats versus the normal rats ($P < 0.01$). And chondrocytes were reduced in volume, arranged loosely and chromatin condensed. However, polydatin reduced the TUNEL positive cell rate and ameliorated cell morphology significantly ($P < 0.01$). It implied that polydatin inhibited the chondrocytes apoptosis in joint tissues of KOA rats.

Polydatin activated the autophagy of chondrocytes in KOA rats

In order to detected the relation between polydatin and autophagy, Western blotting assay was performed to detected the level of autophagy-related proteins. As shown in Figure 3, the ratio of LC3II/LC3I was decreased in the KOA rats ($P < 0.01$), while Beclin-1 expression was markedly decreased compared with the control rats ($P < 0.05$). On the other hand, polydatin reduced the LC3II/LC3I ratio and improved Beclin-1 expression ($P < 0.01$).

In vitro

The effect of polydatin on the proliferation of C28/I2 cells

To explore the effect of polydatin in osteoarthritis pathogenesis, MTT experiment was processed. The viability of C28/I2 cells induced by LPS was much lower than that of normal C28/I2 cells ($P<0.01$), while polydatin evidently promoted the proliferation of C28/I2 cells induced by LPS ($P<0.01$). It indicated that polydatin significantly ameliorated the proliferation of C28/I2 cells (Figure 4A,B).

The effect of polydatin on the apoptosis of C28/I2 cells

The C28/I2 cells from the control, LPS and LPS+polydatin groups were estimate using flow cytometry. Compared with control C28/I2 cells, the proportion of apoptotic cells in the LPS induced C28/I2 cells was obviously increased ($P<0.01$), while that was significantly reduced after treated with polydatin ($P<0.01$) (Figure 4C,D).

The effect of polydatin on the autophagy of C28/I2 cells

We also detected the relation between polydatin and autophagy *in vitro* by Western blotting assay. Consistent with the results *in vivo*, compared with the normal C28/I2 cells group, the ratio of LC3II/LC3I was also decreased and the expression of Beclin-1 was obviously decreased in the C28/I2 cells induced by LPS ($P<0.01$ & $P<0.05$). Compared with the C28/I2 cells induced by LPS, the LC3II/LC3I ratio was markedly improved and the expression of Beclin-1 was increased in C28/I2 cells treated with polydatin ($P<0.01$, Figure 5A~C).

The effect of polydatin on the AMPK/mTOR signaling pathways

mTOR acts a key role in molecule regulating cell autophagy. In order to detected the mechanism of the activation of polydatin on autophagy in C28/I2 cells, we detected the protein expression of mTOR and AMPK by Western blotting. As shown in the Figure 5D~F, the expression of p-AMPK was reduced in LPS group compared with normal group ($P<0.05$), while that of p-mTOR was increased ($P<0.05$). When LPS group cells treated with polydatin, the expression of p-AMPK was obviously increased and that of p-mTOR was obviously decreased respectively ($P<0.01$). It implied that polydatin might regulate autophagy through the AMPK/mTOR signaling pathways.

Dorsomorphin 2HCl blocked the improvement of polydatin on the LPS-induced C28/I2 cells

To further determine whether polydatin improved LPS-induced C28/I2 cells through AMPK/mTOR signaling pathways. The inhibitor of AMPK (Dorsomorphin 2HCl) was used to block the activation of AMPK/mTOR signaling pathways. As shown in Figure 6, after co-treatment with polydatin and Dorsomorphin 2HCl, the promotion of polydatin on the proliferation of C28/I2 cells was reduced, while the inhibition of polydatin on the apoptosis of C28/I2 cells was blocked. Meanwhile, compared with the C28/I2 cells treated with polydatin, the LC3II/LC3I ratio and the expression of Beclin-1 was markedly decreased in C28/I2 cells ($P<0.01$, Figure 7A~C). It means that the activation of autophagy was significantly inhibited by Dorsomorphin 2HCl. What's more, Western blotting results showed the expression of p-mTOR was improved and that of p-AMPK was decreased compared with LPS+polydatin

group ($P<0.05$, $P<0.01$) (Figure 7D~F). It further confirmed that polydatin regulate autophagy through the AMPK/mTOR signaling pathways.

Discussion

KOA is the commonest chronic arthropathy characterized of lost cartilage, formation of osteophyte and sclerosis of subchondral bone, it is further resulting in disability, pain and affect the quality of life [16]. Polydatin can regulate the autophagy by AMPK/mTOR signaling pathway. The molecular mechanisms of polydatin in KOA rat models and C28/I2 cells model was explored in this study. The results showed that polydatin activated autophagy and inhibited apoptosis by AMPK/mTOR signaling pathway.

Cartilage is consist of chondrocytes and is the major component of synovial joints. And chondrocytes have positive effect on the regeneration of hyaline cartilage [17]. In KOA, there are lots of physiological changes in the synovial membrane, cartilage, and bone structures, such as the rough surface of cartilage, disordered chondrocytes and fracture [18]. In this study, we found that polydatin can ameliorate the physiological changes in cartilage tissues of KOA rats according to the HE staining.

In our study, the apoptotic rate was both increased in the cartilage of KOA rats and LPS-induced C28/I2 cells. After treated with polydatin, the apoptotic rate was decreased both *in vivo* and *in vitro*. In addition, the proliferation of C28/I2 cells were also significantly increased after treated with polydatin. For OA patients, the incidence of apoptosis in chondrocytes is much higher than normals [19], and the severity of apoptosis is related to the degree of articular cartilage destruction [20]. Netherlands did a large sample microarray study and found that the expression of apoptosis-related molecules in hematocyte of OA patients were improved [21]. And the same thing, we also confirmed that the key effect of chondrocyte apoptosis in the progress of KOA, and polydatin reduced the apoptosis of chondrocyte and C28/I2 cells.

On the other hand, in KOA chondrocytes, a large number of alterations were observed in various homeostatic mechanisms, such as autophagy and mitochondrial function reduced, and reactive oxygen species (ROS) production increased [22]. Autophagy is a protective energy-conserving mechanism, and it transfers dysfunctional organelles or macromolecules into their constituent molecules during cell stress and starvation [23]. During autophagy, newly formed double-membraned vesicles envelope dysfunctional protein to make up autophagosomes, then it is fused with lysosomes and resulting in lysosomal breakdown [24]. Compared with non-OA control, the activity of autophagy pathways in the cartilage of OA patients was inhibited, and it means that autophagy pathways might related to the pathogenesis of OA cartilage [25]. Our results showed that the expression of autophagy-related proteins and autophagic activity in the articular cartilage of KOA rats and C28/I2 cells was lower than that in the normals. However, the activity of autophagic was activated by polydatin treatment. In OA cartilage and chondrocytes, the expression of autophagy-related proteins (Beclin1 and LC3II/I) was decreased, and the levels of the signaling factors related to apoptotic were improved [26]. Therefore, we hypothesized that the damaged chondrocytes could self-protection by inhibit the autophagy.

Crucial molecules and pathways of AMPK/mTOR have been shown to regulate autophagy and received the most attention from researchers [27]. mTOR could improve the autophagy activity and reduce the apoptosis of chondrocytes in a KOA, further reduce the degree of OA [28]. It has been proved that polydatin regulated important molecules involved in autophagy and inflammation signaling pathways in bovine mammary epithelial cells [29], acute kidney injury [30, 31] and myocardial injury in diabetic rats [32, 33]. In this study, it was found that the effects of polydatin on autophagy induction was mTOR-dependent and regulated by AMPK. In addition, the activation of polydatin on autophagy was blocked by the inhibitor of AMPK (Dorsomorphin 2HCl). These results revealed that the activation of chondrocyte autophagy induced by polydatin can inhibit apoptosis, and the mechanism might related to AMPK/mTOR signaling pathway.

In conclusion, by establishing a KOA model both in vivo and in vitro, we investigated the possible effect of polydatin for the treatment of KOA. The results demonstrated that polydatin treatment could ameliorate the pathological damage and reduce the chondrocyte apoptosis in cartilage tissues of KOA rats; increase the cell activity, inhibite apoptosis and activate autophagy of LPS-stimulated C28/I2 cells. At the same time, we found that polydatin displayed the pro-autophagic activity in KOA via the AMPK/mTOR pathway. These findings suggested polydatin as a potential therapeutic agent for KOA.

Abbreviations

KOA	Knee osteoarthritis
SD	Sprague-Dawley
HE	Hematoxylin-eosin
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
RIPA	Radioimmunoprecipitation assay
PVDF	polyvinylidene difluoride
ECL	Enhanced chemiluminescence
ROS	reactive oxygen species

Declarations

Acknowledgments

Not applicable.

Authors' contributions

Zhengcong Ye and Chun He designed the research and acquisition of data, Pengzheng Yu and Guoping Cao analyzed the data. Canfeng Wang drafted the manuscript. Qinrong Shen revised the manuscript for

important intellectual content.

Data availability statement

All data generated or analyzed during this study are included in this article.

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Ethics approval and consent to participate

The animal experiments were in accordance with the guidelines of laboratory animal care and were approved by the Hangzhou Eyong Biotechnological Co., Ltd. Animal Experiment Center (Hangzhou, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

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Figures

Figure 1

Polydatin ameliorate the pathological changes of cartilage tissues in KOA rats. A, HE staining images in cartilage tissues of each group rats (original magnification 100×, 200×). B, Semi-quantitative analysis of the histological lesions. ($\bar{x} \pm s$, n=10), ▲P<0.05, ▲▲P<0.01 vs. control, ▨P<0.05, ▨▨P<0.01 vs. KOA.

Figure 2

Polydatin inhibit the apoptosis of chondrocytes in joint tissues of KOA rats. A, TUNEL staining images in rats' joint tissues (original magnification 200×, 400×); B, Semi-quantitative assessment of TUNEL positive cell rate in rats' joint tissues. ($\bar{x} \pm s$, n=10), ▲P<0.05, ▲▲P<0.01 vs. control, ▨P<0.05, ▨▨P<0.01 vs. KOA.

Figure 3

Expression of autophagy-related proteins LC3 and Beclin-1 in the cartilage tissue after treated with polydatin. A, Representative immunoblots of LC3 and Beclin-1 proteins in different treatment groups. B and C, Quantitative densitometric analysis of LC3 and Beclin-1. ($\bar{x} \pm s$, $n=3$), ▲ $P<0.05$, ▲▲ $P<0.01$ vs. control, ▢ $P<0.05$, ▢▢ $P<0.01$ vs. KOA.

Figure 4

The viability and apoptotic of C28/I2 cells was detected by MTT assays and flow cytometry. A and B, the OD value and survival rate in control, LPS and LPS+polydatin group. C and D, flow cytometry detected the

apoptotic of C28/I2 cells in control, LPS and LPS+polydatin group. ($\bar{x}\pm s$, n=3), ▲P<0.05, ▲▲P<0.01 vs. control, ▨P<0.05, ▨▨P<0.01 vs. LPS.

Figure 5

Polydatin ameliorated articular cartilage degeneration might occur through autophagy regulation mediated by the AMPK/mTOR signaling pathway. A and D, Representative immunoblots of each protein in different treatment groups. B, C, E and F, Quantitative densitometric analysis of LC3, Beclin-1, p-AMPK, AMPK, p-mTOR and mTOR. ($\bar{x}\pm s$, n=3), ▲P<0.05, ▲▲P<0.01 vs. control, ▨P<0.05, ▨▨P<0.01 vs. LPS.

Figure 6

The effect of polydatin and Dorsomorphin 2HCl on the viability and apoptotic analysis of C28/I2 cells. A and B, the OD value and survival rate in control, LPS, LPS+polydatin and LPS+polydatin+Dorsomorphin 2HCl group. C and D, flow cytometry detected the apoptotic of C28/I2 cells in control, LPS, LPS+polydatin group and LPS+polydatin+Dorsomorphin 2HCl group. ($\bar{x} \pm s$, $n=3$), ▲ $P<0.05$, ▲▲ $P<0.01$ vs. control, □ $P<0.05$, □□ $P<0.01$ vs. LPS, # $P<0.05$, ## $P<0.01$ vs. LPS+polydatin.

Figure 7

The improvement of polydatin on articular cartilage degeneration through autophagy regulation mediated by the AMPK/mTOR signaling pathway was blocked by Dorsomorphin 2HCl. A, D. Representative immunoblots of each protein in different treatment groups. B, C, E and F, After different treatment, the expression of LC3, Beclin-1, p-AMPK, AMPK, p-mTOR and mTOR was verified by western blot in each group. ($\bar{x} \pm s$, $n=3$), $\blacktriangle P<0.05$, $\blacktriangle\blacktriangle P<0.01$ vs. control, $\boxtimes P<0.05$, $\boxtimes\boxtimes P<0.01$ vs. LPS, $\#P<0.05$, $\#\#P<0.01$ vs. LPS+polydatin.