Emodin Ameliorate High Glucose-induced Podocyte Apoptosis via Regulating AMPK/mTOR- mediated Autophagy Signaling Pathway

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Research Article

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Abstract

Background: Podocyte apoptosis and autophagy dysfunction have been considered to be one of the important causes of diabetic nephropathy (DN). Emodin has the function of regulating autophagy. The present study was performed to investigate the effect of emodin on high glucose (HG)-induced podocyte apoptosis and whether the potential anti-apoptotic mechanism of emodin is related to the induction of AMPK/mTOR-mediated autophagy in MPC5 cells in vitro.

Methods: The viability and apoptosis of podocytes (MPC5 cells) were detected using CCK-8 assay, trypan blue exclusion assay and flow cytometry analysis, respectively. The expression levels of Cleaved caspase-3, autophagy maker LC3 I/II, and AMPK/mTOR signaling pathway-related proteins were evaluated with western blot analysis. The changes of morphology and RFP-LC3 fluorescence were observed under microscopy.

Results: HG (20-160 mmol/L) dose-dependently induced cell apoptosis in MPC5 cells, whereas emodin (4 μmol/L) significantly ameliorated HG-induced cell apoptosis and caspase-3 cleavage. Emodin (4 μmol/L) significantly increased LC3-II levels and induced RFP-LC3-containing punctate structures in MPC5 cells. Furthermore, the protective effects of emodin were mimicked by rapamycin (100 nmol/L). Moreover, emodin increased the phosphorylation of AMPK and suppressed the phosphorylation of mTOR. The AMPK inhibitor compound C (10 μmol/L) abolished emodin-induced autophagy activation.

Conclusion: Emodin ameliorated HG-induced apoptosis of MPC5 cells in vitro that involved induction of autophagy through the AMPK/mTOR signaling pathway, which might provide a potential therapeutic option for DN.

Background

Diabetic nephropathy (DN) is a diabetes-induced microvascular complication which has become the primary cause of end-stage renal failure [1]. Progressive proteinuria is a significant clinical feature of DN caused by an impaired glomerular filtration barrier [2]. Podocytes are highly differentiated glomerular epithelial cells, which together with glomerular basement membrane (GBM) and vascular endothelial cells constitute glomerular filtration barrier [3]. Glomerular podocyte injury and apoptosis play an important role in the progression of DN, especially in the formation of proteinuria and glomerulosclerosis [4].

Autophagy is an important cellular process that involved in the maintenance of cell renewal and homeostasis through the degradation of lysosomal proteins and the removal of damaged structures or overexpressed proteins [5]. Autophagy pathway can be activated under the stress conditions of nutrition deficiency, ischemia and hypoxia, oxidative stress, etc. Research has shown that the basic level of autophagy in podocytes is significantly higher than that in other kidney proper cells, and this high level of autophagy is necessary to maintain the normal physiological function of podocytes [6]. The high level of autophagy activity of podocytes is conducive to the degradation or removal of damaged proteins and aging organelles, so as to maintain cell homeostasis. Apoptosis is a programmed method of gene

regulation and biological autonomy in order to maintain a constant number of cells. Recent studies have demonstrated that autophagy is closely related with apoptosis in the development and progression of DN [7, 8]. Apoptosis of podocytes is present in early stage of DN, and autophagy activity is significantly increased when the podocytes are damaged [9]. Therefore, exploring the relationship between podocyte autophagy and apoptosis will provide an important therapeutic strategy for drug treatment of DN.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is an active anthraquinone constituent extracted from the rhizome of rhubarb Rheum officinale Baill. [10]. It has been shown that emodin possesses various pharmacological properties, including anti-bacterial [11], anti-inflammation [12], immunosuppressive [13], antiproliferation [14], anticancer [15] and antioxidant activities [16]. Rhubarb preparations have been widely used in clinical treatment of DN. Previous studies have shown that the mechanism of emodin in the treatment of DN may be related to the inhibition of cell proliferation [17] and inflammatory response [18]. Recent studies have shown that emodin can improve the damage of DN by regulating autophagy signaling pathway [16, 17]. The pathogenesis of DN is related to nutrition sensitive pathways such as AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) [18], autophagy is positively regulated by AMPK, but negatively regulated by mTOR [19]. AMPK activation leads to the phosphorylation and activation of TSC1/2 complex, which can indirectly inhibit the activity of mTOR by inhibiting the activity of rheb enzyme. It can also directly phosphorylate a subunit of mTORC1, raptor, to inhibit mTOR and enhance autophagy. It has been reported that emodin is an effective AMPK activator [20], and can also regulate mTOR pathway [21]. In our vivo study shows that emodin ameliorates podocyte injury in DN rats by regulating AMPK/mTOR-mediated autophagy signaling pathway [22]. In order to further verify the protective effect of emodin on podocytes of DN, we conducted experiments in vitro.

In this study, we investigated the effects and molecular mechanisms of emodin on podocyte injury induced by high glucose (HG). Our data suggest that emodin might ameliorate HG-induced podocyte apoptosis by regulating the AMPK/mTOR-mediated autophagy signaling pathway. These results provide evidence for the emodin in the treatment of DN.

Methods

Reagents

Emodin (E7881), rapamycin (Rap, CAS#: 53123-88-9) and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co (St Louis, MO, USA). Dorsomorphin (Compound C, S7840) was purchased from Selleck Chemicals LLC (Houston, Texas, USA). Recombination γ-Interferon (IFN-γ) was purchased from Pepro Tech Inc (Rocky Hill, New Jersey, USA). D-glucose (HG) was purchased from MedChemExpress (New Jersey, USA). Emodin was dissolved in DMSO to a concentration of 10 mmol/L.

Cell culture

The conditioned immortalized mouse podocytes (MPC5) were kindly provided by Professor Jun Yuan, Department of Nephrology, the Affiliated Hospital of Hubei University of Chinese Medicine, cells were from Professor Peter Mundel Laboratory (Mount Sinai Medical Center, New York, USA). Undifferentiated podocytes were cultured in RPMI 1640 medium (Hyclone, Thermo Fisher, Beijing, China) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) containing 10 U/ml IFN-γ, 100U/mL penicillin G and 100μg/mL streptomycin (Gibco BRL) at 33℃ under an atmosphere of 5% CO_2, and induced to differentiate supplemented with 10% FBS without IFN-γ at 37℃ and 5% CO_2 for 10-14 days in RPMI-1640 medium. The differentiated podocytes were used in subsequent experiments.

Evaluation of viable cells

The number of viable cells was assessed by trypan blue exclusion. Cells were treated with the indicated condition, then collected and resuspended in the trypan blue solution (0.4%), finally counted under a light microscope with a hemacytometer. At least three independent experiments were conducted.

Cell Proliferation Assay

The Cell Counting Kit-8 (CCK-8, Beyotime Biotechnology, Shanghai, China) was used to detect cell viability according to the manufacturer's instructions. The differentiated MPC5 cells (5×10^3 cells per well) were seeded into 96-well plates and incubated with 5% CO_2 at 37 ℃. When the cells proliferated to 70%-80% fusion in the plate, we sequentially changed to different concentrations of HG medium to each group. CCK-8 and serum-free RPMI 1640 medium were mixed at a ratio of 1:10 after 48 h, and then the cells were incubated for 2 h. The absorbance values of each well were measured at 450 nm using a microplate reader (SpectraMax i3x, Molecular Devices, Shanghai, China).

Flow cytometric (FACS) analysis of apoptosis

The apoptosis of MPC5 cells was assessed by using flow cytometry (Becton Dickinson) to analyze Annexin V-FITC and PI-stained cells labeled using the Annexin V-FITC Apoptosis Detection Kit (KeyGen Biotech, Nanjing, China) according to the manufacturer's protocol. Briefly, cells were treated with the indicated condition, and then collected and resuspended in binding buffer. Subsequently, cells were incubated with Annexin V-FITC (5 μl) and PI (5 μl) for 15 min [23]. The percentage of Annexin V-FITC and PI stained cells was assessed using Accuri C6 software (Becton Dickinson).

Western blot analysis

Podocytes cells were collected and lysed with RIPA lysis buffer (Beyotime, Hainan, China). Samples were obtained via centrifugation at 13,000g and 4°C for 5 min. The supernatants were boiled at 100°C for 5

min in loading buffer. Lysate protein concentrations were determined by bicinchoninic acid (BCA) protein concentration assay kit (Beyotime Biotechnology, Shanghai, China). Equal amounts of protein were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrophoretically transferred to polyvinylidene fluoride membrane (Millipore, Bedford, USA). The membranes were blocked with 5% skimmed milk at room temperature for 1 h, and then incubated overnight at 4 ℃ with primary antibody as follows: anti-rat AMPK (ab80039), anti-rat p-AMPK (ab23875), anti-rat β-actin (ab227387) antibodies were from Abcam Ltd, HKSP, New Territories, HK; anti-rat LC3 I/II (12741), anti-rat mTOR (2983), anti-rat p-mTOR (5536), anti-rat Cleaved caspase-3 (9661) antibodies were from Cell Signaling Technology Company, Beverly, MA, USA; anti-rat caspase-3 (BM4620) antibodies were from Boster Biological Technology Co., Ltd, Wuhan, China; Horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulins and anti-mouse immunoglobulins (KPL Company, USA) were used as the secondary antibody. The membranes were coated using HRP-labeled chemiluminescent substrates (Millipore, Bedford, USA), eventually exposed and fixed in the dark box. This procedure was carried out 3 times. The results were quantified using Image-Pro Plus 6.0 software (Media Cybernetic, Washington, USA), which were contrasted with densitometric signal of β-actin, respectively, and the ratios were expressed as the relative protein contents.

Transient transfection

MPC5 cells were transfected with the pmRFP-LC3 plasmid using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The pmRFP-LC3 (#21075) plasmid was obtained from Addgene. MPC5 cells transfected with pmRFP-LC3 were then incubated with or without emodin for 1 h and observed for the formation of autophagosomes under a fluorescent microscope.

Statistical analysis

The data were analyzed using statistical software SPSS 24.0. Significant differences were evaluated using one-way ANOVA with Bonferroni post-hoc test (GraphPad Prism 6.0, La Jolla, CA, USA). A *P*-value < 0.05 was considered to be statistically significant.

Results

High glucose induces apoptosis in podocytes

Previous studies have suggested that high glucose (HG) could induce apoptosis in renal podocytes [24, 25]. In our study, firstly, MPC5 cells were exposed to different concentrations of HG (from 2.5 to 160 mmol/L) for 48 h, cell proliferation rate was detected. The results of the CCK-8 assay showed that the proliferation rate of cells was gradually decreases when HG concentration is higher than 5 mmol/L (Fig. 1a). Secondly, MPC5 cells were treated with different concentrations of HG (from 20 to 160 mmol/L) for

48 h, and then cell viability was evaluated. As expected, HG resulted in concentration-dependent cell apoptosis, cells treated with 40 mmol/L HG showed obviously apoptosis (Fig. 1b). These data indicate that HG induced MPC5 cell apoptosis in a dose-dependent manner.

We also examined the expression of apoptosis-related markers after exposing cells to 40 mmol/L HG for different time intervals. Western blot showed that the expression of Cleaved caspase-3 increased when cells were treated with HG after 12 h (Fig. 1c). Additionally, after 48 h of treatment, the results of flow cytometry revealed that an increased fraction of apoptotic HG-treated cells (11.4% early apoptotic cells and 1.69% late apoptotic cells) was detected (Fig. 1d). Taken together, these data confirmed that the pro-apoptotic effect of HG on MPC5 cells was related to HG concentration and treatment time.

Effect of emodin on podocyte apoptosis induced by high glucose

To examine whether HG-triggered podocyte apoptosis can be inhibited by emodin, MPC5 cells were incubated in medium containing normal glucose (5.5 mmol/L), 40 mmol/L HG or 40 mmol/L HG plus different concentrations of emodin for up to 48 h,

microscopic analysis was performed and the viable cells were tested. The results indicate that the HG-treated cells exhibited abundant cellular apoptosis that was markedly attenuated by treatment with emodin at 4 μmol/L concentration (Fig. 2a). Under phase-contrast microscopy (magnification, ×100), compared with the normal glucose group, 40 mmol/L HG-treatment decreased the number of viable cells, which was obviously reversed together with 4 μmol/L emodin treatment (Fig. 2b).

Additionlly, the anti-apoptotic effect of emodin was further confirmed by western blot analysis, experimental results indicated that HG treatment in MPC5 cells induced the level of Cleaved caspase 3, while intervention with emodin significantly suppressed the expression of Cleaved caspase-3 (Fig. 2c). Taken together, these results indicate that emodin could effectively protect against HG-induced apoptosis in MPC5 cells.

Induction of autophagic activity by emodin in MPC5 cells

Autophagy plays a critical role in maintaining cell homeostasis and might serve as an anti-apoptotic mechanism. Therefore, we examined the effects of HG and emodin on the expression of autophagy biomarkers, including microtubule-associated protein 1 light chain 3 (LC3) and it lipidated form (LC3-II) [26]. When MPC5 cells were exposed to 40 mmol/L HG at different time points, LC3-II markedly increased and reached a maximum level at 1 h (Fig. 3a). We also examined whether autophagy could be induced in MPC5 cells exposed to emodin at different time points. The results showed that treatment with emodin can also increased the level of LC3-II, which reached a maximum level at 1 h, and then at 6 h (Fig. 3b).

In order to further verify emodin effectively induced autophagic activation in MPC5 cells, we selected another common method to detect autophagy, which is the RFP-LC3 labeling method. MPC5 cells were transiently transfected with RFP-LC3 and then incubated in medium containing emodin. Under the fluorescence microscope, we observed that cells treatment with emodin contained an increased number of bright fluorescent particles at 1 h, whereas cells without emodin treatment showed a diffuse distribution of red fluorescence (Fig. 3c)., which indicated an increase in the formation of autophagosomes. HG could induce autophagy [27], which may exert protective effects against HG-induced apoptosis. In our experiment, emodin also exhibited analogous effect. We speculate that emodin's inhibition of podocyte apoptosis induced by HG may be related to this protective mechanism.

Autophagy protects podocytes from apoptosis induced by high glucose

In order to verify the protective effect of autophagy on podocyte apoptosis induced by high glucose, we selected rapamycin, the activator of autophagy, which has been reported to activate autophagy by inhibiting mTOR signaling pathway [28]. As expected, western blot analysis showed that autophagic activity was significantly induced by rapamycin treatment at different time points, especially at 1 h and 6 h time point (Fig. 4a). Subsequently, cells were exposed to HG in the absence or presence of rapamycin for 48 h and were subjected to morphological observation, we observed that rapamycin treatment can significantly attenuate the apoptosis induced by HG (Fig. 4b). Additionally, cells were similarly exposed to HG with or without rapamycin for 48 h and were subjected to FACS analysis. As shown in Fig. 4c, FACS analysis revealed that approximately 12.15% early apoptotic and 4.42% late apoptotic cells were detected in HG-treated MPC5 cells. However, in the rapamycin-treated group, the percentages of early and late apoptotic cells dropped to approximately 6.36% and 3.19%, respectively. Finally, western blot analysis showed that emodin and rapamycin could suppress caspase-3 cleavage or activation during HG treatment (Fig. 4d), supporting the cytoprotective effect of emodin and rapamycin. These data demonstrate that autophagy plays a protective role in podocyte apoptosis induced by high glucose, emodin may have anti-apoptotic effect similar to rapamycin.

Emodin induces autophagy by regulating the AMPK/mTOR signaling pathways

To identify the mechanism by which emodin induces autophagy, we examined the effects of emodin on AMPK activity and mTOR signaling, both of which are well-known upstream regulators of autophagy. When MPC5 cells were exposed to emodin at different time points, the phosphorylation of AMPK was significantly increased, especially at 1 h time point, while the phosphorylation of mTOR was markedly suppressed, which also most obviously at 1 h time point (Fig. 5a). Subsequently, compound C, a well-known AMPK inhibitor, was used in our experiment. As shown in Fig. 5b, when MPC5 cells were exposed to emodin, pmRFP-tagged LC3-transfected cells exhibited increased punctate structures, while these

number of punctate structures was significantly decreased when cells were treatment together with compound C. Western blot showed that emodin significantly increased the ratio of LC3-II/LC3-I, which was significantly down regulation when compound C was added (Fig. 5c). These data suggested that emodin increased podocyte autophagy possibly through regulating AMPK/mTOR signaling pathways under high glucose treatment.

Discussion

DN has now gradually become a major cause of end stage renal disease (ESRD). However, the completely effective treatment is still limited at present. Increasingly studies show that traditional Chinese medicine treatment can delay the progression of DN, and its mechanisms are varied. In the present investigation, we found that emodin, a bioactive substance found in rhubarb, increased autophagy and suppressed HG-induced podocyte apoptosis. Therefore, we speculated that emodin might exert protective effects via inhibiting podocyte apoptosis and promoting cell autophagy in DN.

Podocytes are highly differentiated glomerular epithelial cells which located on the surface of GBM, and play a key role in maintaining the structure and function of the glomerular filtration barrier. The loss and impairment of podocytes is major cause of nephrotic proteinuria and glomerular sclerosis, and is related to the initiation and progression of DN [4]. A certain concentration of HG can induce podocyte apoptosis, in our experiment, we observed that 40 mmol/L HG can significantly induce podocyte apoptosis. Meanwhile, the expression of pro-apoptotic protein Cleaved caspase-3 was significantly increased. After emodin intervention, HG-induced podocyte apoptosis was significantly reversed, and the expression of Cleaved caspase-3 was markedly inhibited, which indicates that emodin has protective effect on podocyte injury.

Autophagy participates in organelle metabolism and bioenergy supply through degrades long-lived proteins and organelles, and then to maintain the stability of the cell environment [29]. Under normal physiological conditions, the basic level of autophagy exists in almost all cells and plays an important role in cell growth, proliferation and death. Studies have reported that in the pathophysiological process of the kidney, autophagy is closely related to the intrinsic cells of the kidney, such as podocytes and renal tubular epithelial cells [30, 31]. There are also studies showed that autophagy has appeared in diabetic kidney injury [32], renal ischemia-reperfusion injury [33], and toxic kidney injury [34], indicating that autophagy may be involved in a variety of kidney diseases and plays an important role. Under normal conditions, podocytes maintain a certain level of autophagy; in our experimental results, we observed a few autophagosomes were found in podocytes cultured with basic concentration of HG (5.5 mmol/L). LC-3, known as microtubule associated protein light chain 3, is synthesized in cells and located in the cytoplasm. In the process of autophagy, LC-3 type I is modified by ubiquitin like system, covalently combined with phosphatidylethanolamine, and located on the autophagosome membrane to form LC-3 type II. The inversion of the relative expression ratio of type I and type II can be used to indicate the activity of autophagy [35, 36]. In this experiment, western blotting showed that the ratio of LC3-II/LC3-I was significantly increased at 1 h and 6 h time point after HG and emodin treatment, indicating that

autophagy activity was enhanced. So we speculate that autophagy has a self-stabilizing effect and plays a protective role in podocyte damage, and emodin may reduce HG-induced podocyte apoptosis by enhancing autophagy.

Autophagy is regulated by two main nutrient-sensing pathways, mTOR and AMPK [37]. As we all known, mTOR is a target protein of rapamycin, which can regulate cell growth and autophagy. In the condition of adequate nutrition or without stress, mTOR is activated and autophagy is inhibited; however, mTOR activity is inhibited and autophagy pathway is activated when the cells are in a stress state or starvation environment under nutritional deficiency [38]. Under stress conditions, rapamycin can specifically bind to mTOR and inhibit the protein kinase activity of mTOR, thus inducing autophagy [39]. In this study, rapamycin was used to interfere with MPC5 cells. It was found that rapamycin treatment could increase the ratio of LC3-II/LC3-I, and rapamycin combined with HG could significantly ameliorate HG-induced podocyte apoptosis. Our previous studies have shown that emodin can regulate mTOR pathway [22, 23], in the present experiment, we investigated the effect of emodin on autophagy at different time points (1, 2, 3, 6 h). The results showed that the ratio of LC3-II/LC3-I also increased significantly, which indicated that emodin could induce autophagy analogue to rapamycin. Emodin treatment also increased the autophagy fluorescence granules, which further confirmed that emodin could induce autophagy. AMPK pathway is one of the upstream pathways of mTOR. Activation of AMPK can inhibit mTOR and enhance autophagy. It has been reported that emodin is an effective AMPK activator [20], our results showed that the expression of p-mTOR protein was significantly down-regulated and the expression of p-AMPK was up-regulated with the prolongation of emodin intervention time, which was most obvious at 1 h. Therefore, emodin may induce autophagy in MPC5 cells by regulating AMPK/mTOR signaling pathway. Compound C, which is a well-known AMPK inhibitor [40], when cells were treatment emodin together with compound C, autophagy fluorescence granules increased by emodin was obviously suppressed when added compound C. Similarly, western blotting showed that emodin can increase the ratio of LC3-II/LC3-I, which was reversed by compound C. It was further confirmed that emodin might regulate AMPK/mTOR signaling pathway.

In conclusion, we found that emodin could induce autophagy in HG-treated MPC5 cells and the potential mechanism underlying the protective role of emodin against HG-induced podocyte apoptosis, that involved induction of autophagy through the AMPK/mTOR pathway. This study confirmed that emodin ameliorates HG-induced podocyte apoptosis and provided additional evidence in support of the clinical usage of emodin in the treatment of DN.

Abbreviations

DN: Diabetic nephropathy; HG: High glucose; AMPK: AMP-activated protein kinase; mTOR: Mammalian target of rapamycin; MPC5: Mouse podocyte; CCK-8: Cell Counting Kit-8; GBM: Glomerular basement membrane; LC3: Microtubule-associated protein light chain 3;TSC1/2: Tuberous sclerosis complex gene 1/2; DMSO: Dimethyl sulfoxide; IFN-γ: Recombination γ-Interferon; RPMI: Roswell park memorial institute; FBS: Fetal bovine serum; Annexin V-FITC: Annexin V-Fluorescein Isothiocyanate; PI: Propidium iodide;

FACS: Fluorescence activated cell sorter; RIPA: Radio-Immunoprecipitation assay; BCA: Bicinchoninic acid; SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HRP: Horseradish peroxidase; ESRD: End stage renal disease.

Declarations

Acknowledgments

Not applicable.

Authors' contributions

Conceived and designed the experiments: LH, ZY, CW and XF. Performed the experiments: LH, HY, SG and YW. Analyzed the data: LH, CW, HT and WH. Wrote the manuscript: LH and HY. All authors read and approved the final version of the manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

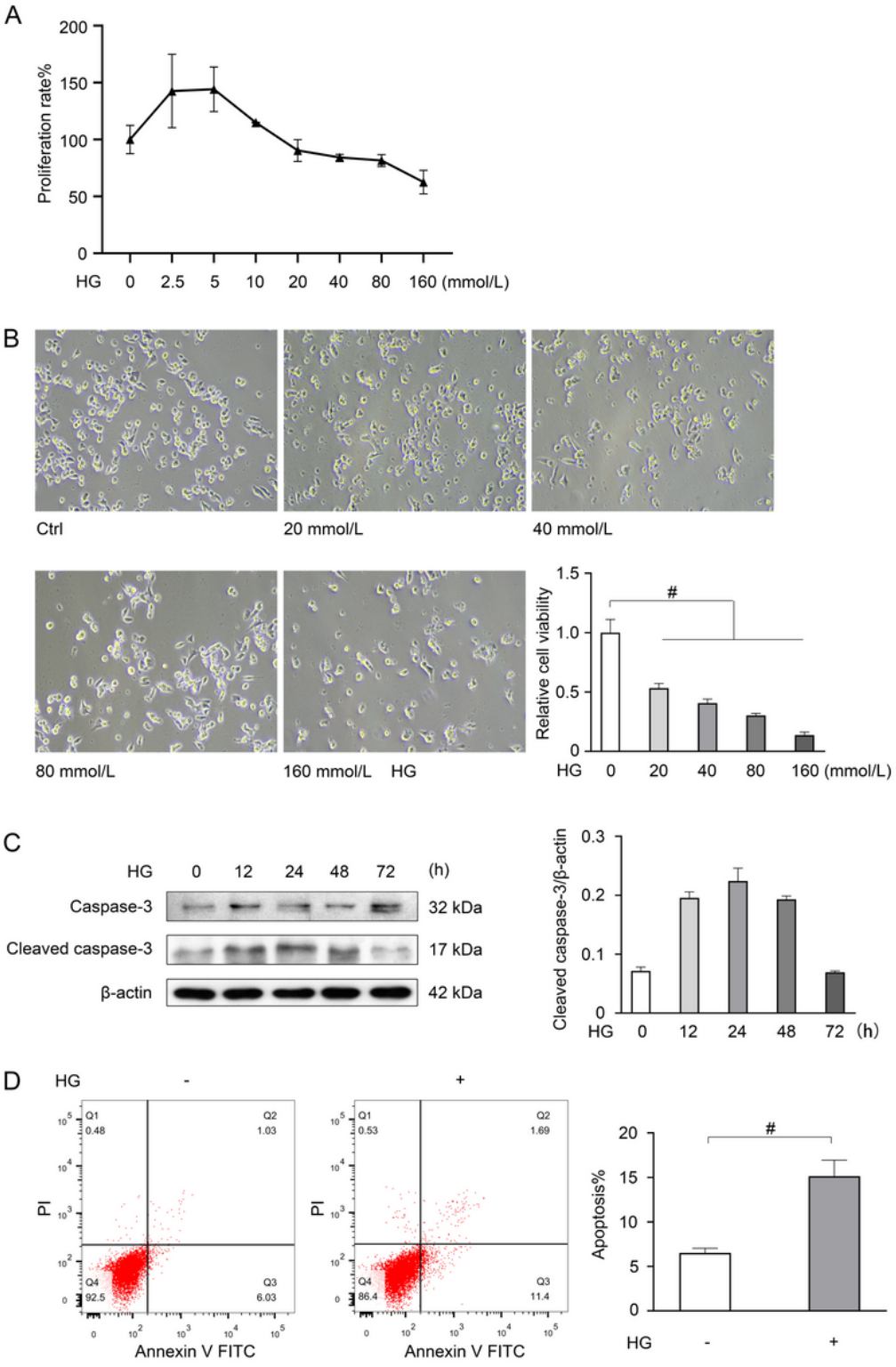

Figure 1

High glucose induces apoptosis in podocytes. a MPC5 cells were treated with different concentrations of HG (2.5–160 mmol/L) for 48 h, cell proliferation rate was detected by CCK-8 assay. b MPC5 cells were incubated in medium containing normal glucose (Ctrl, 5.5 mmol/L) and different concentrations of HG (20–160 mmol/L) for 48 h were subjected to phase-contrast microscopy (magnification, ×200) and an assessment of cell viability by trypan blue exclusion assay. c MPC5 cells were treated with 40 mmol/L

HG at different time points and subjected to Western blot analysis of Caspase-3 and Cleaved caspase-3 expression. The level of β-actin is shown as a loading control and quantitative analysis of Cleaved caspase-3 is presented. d Cells were incubated in medium containing normal glucose (5.5 mmol/L) and 40 mmol/L HG for 48 h were subjected to Annexin V and PI assay and quantification of the percentage of apoptotic cells. #P < 0.01.

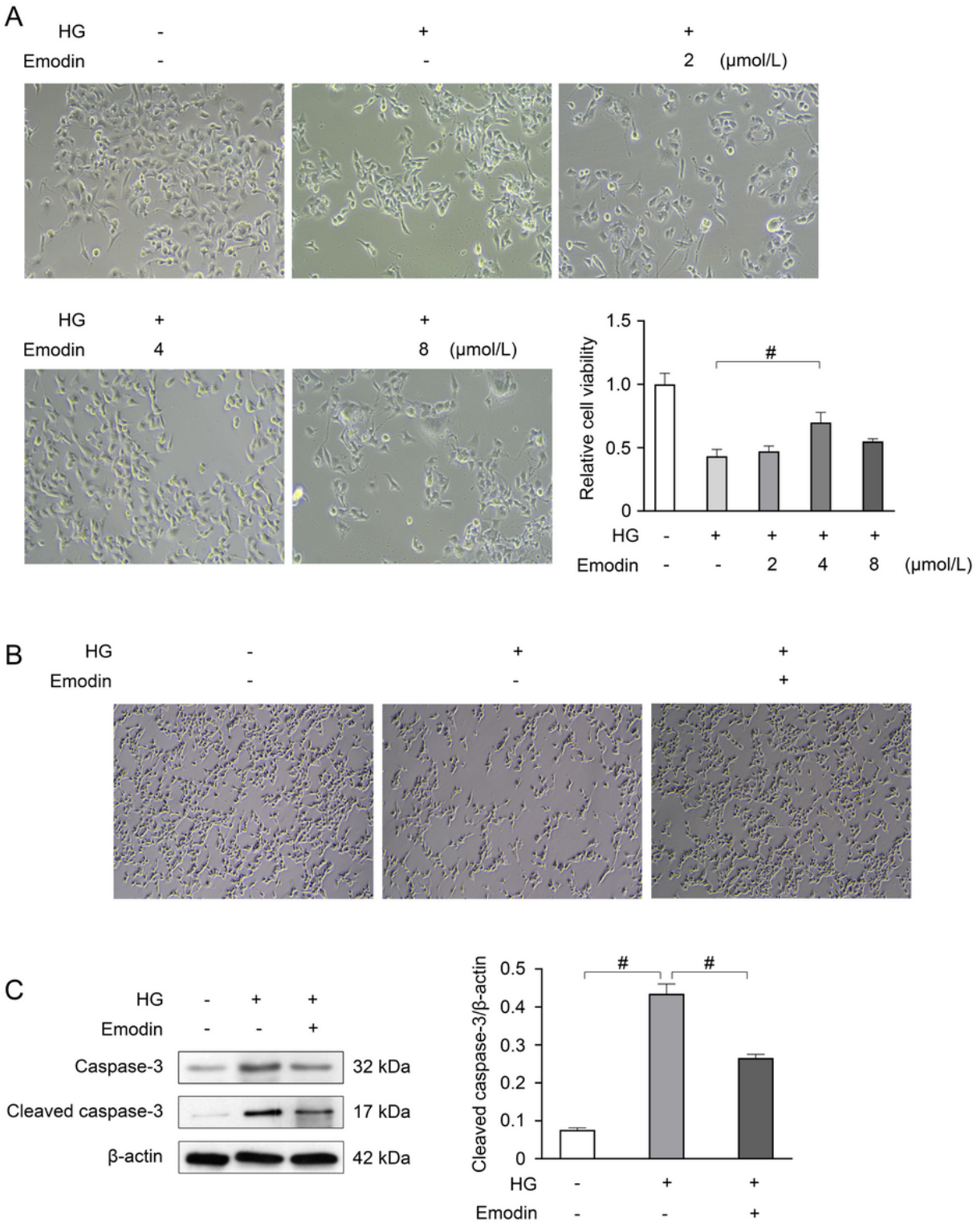

Figure 2

Effect of emodin on podocyte apoptosis induced by high glucose. a MPC5 cells were incubated in medium containing normal glucose (5.5 mmol/L) or 40 mmol/L HG together with the indicated concentrations of emodin for 48 h and were subjected to phase-contrast microscopy (magnification, ×200) and an assessment of cell viability by trypan blue exclusion assay. b MPC5 cells were incubated in medium containing normal glucose (5.5 mmol/L) or 40 mmol/L HG in the absence or presence of 4 μmol/L emodin for 48 h and were subjected to phase-contrast microscopy (magnification, ×100). c Cells were incubated in medium containing normal glucose (5.5 mmol/L) or 40 mmol/L HG with or without 4 μmol/L emodin for 48 h and were subjected to Western blot analysis of Caspase-3 and Cleaved caspase-3 levels. The level of β-actin is shown as a loading control and quantitative analysis of Cleaved caspase-3 is presented. #P < 0.01.

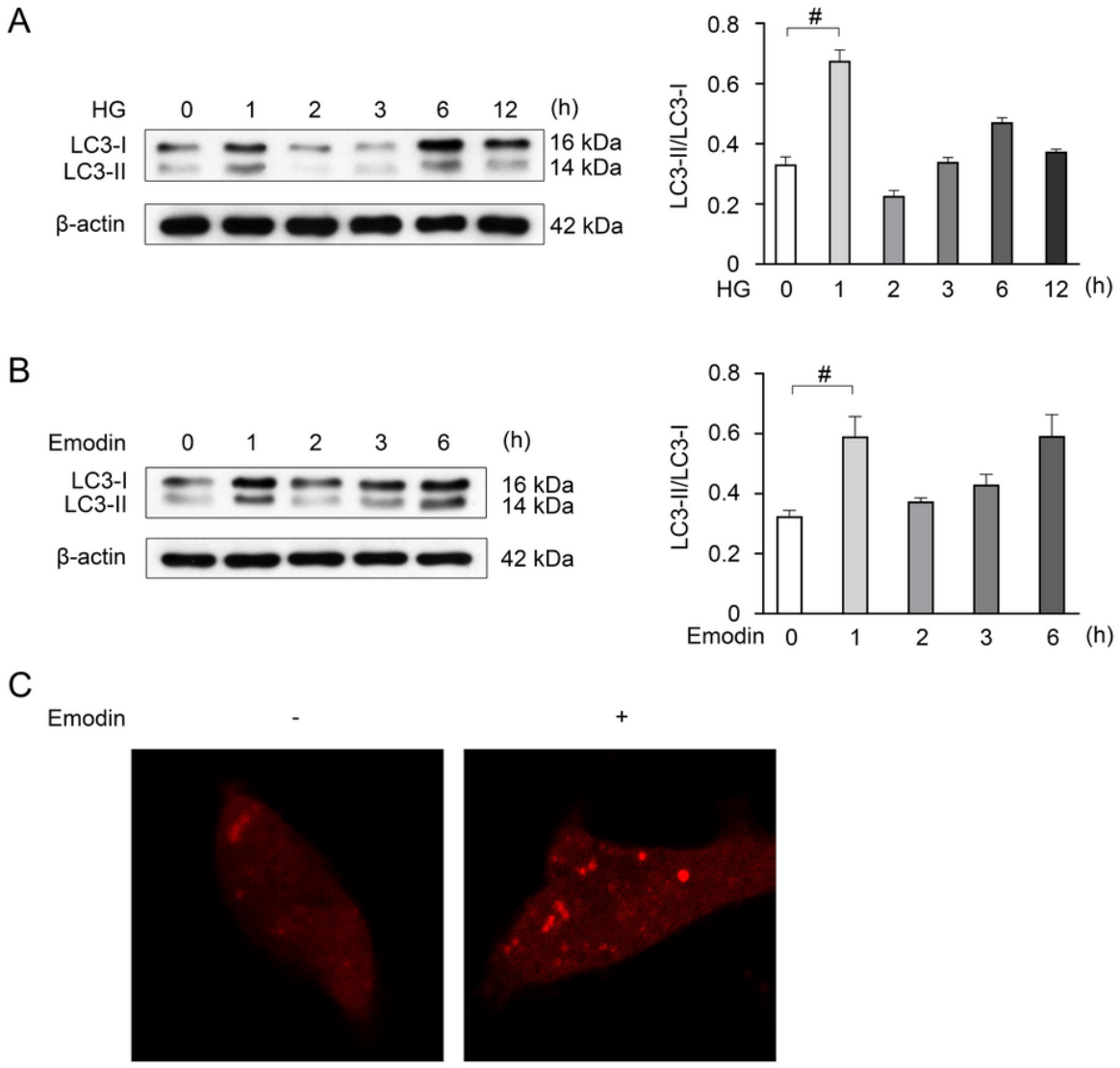

Figure 3

Induction of autophagic activity by emodin in MPC5 cells. a MPC5 cells were treated with 40 mmol/L HG for 0–12 h and subjected to Western blot analysis of LC3-I and LC3-II protein levels. The level of β-actin is shown as a loading control and quantitative analysis of LC3-II is presented. b Cells were treated with 4

μmol/L emodin for 0–6 h and were subjected to western blot analysis of LC3-I and LC3-II protein levels. The level of β-actin is shown as a loading control and quantitative analysis of LC3-II is presented. c Fluorescent microscopic analysis of MPC5 cells transfected with pmRFP fluorescent-tagged LC3 plasmid and exposed to 4 μmol/L emodin for 1 h. The red color indicates autophagosomes. #P < 0.01.

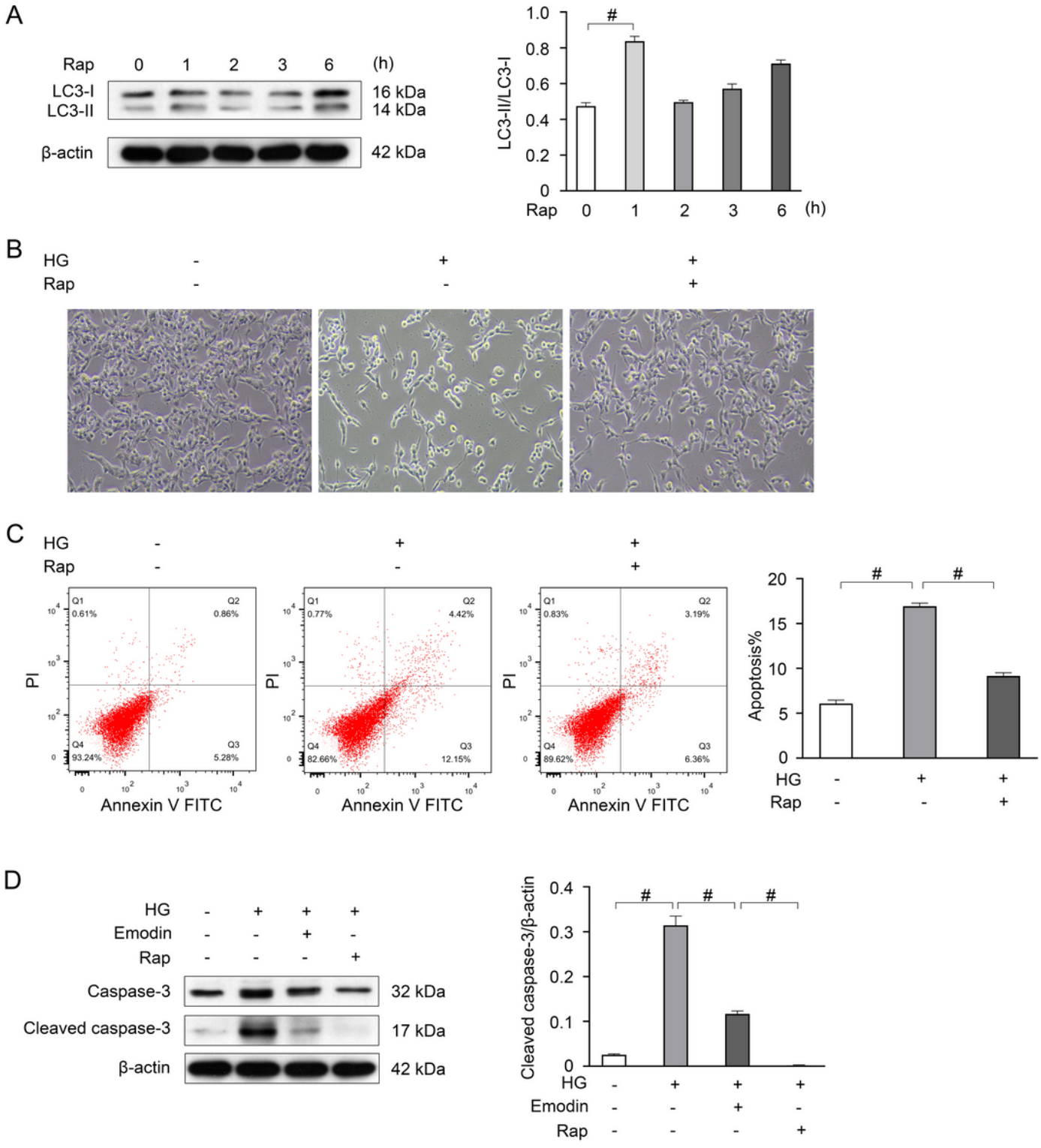

Figure 4

Autophagy protects podocytes from apoptosis induced by high glucose. a MPC5 cells were treated with 100 nmol/L rapamycin (Rap) at different times and were subjected to Western blot analysis of the LC3-I and LC3-II protein levels. The level of β-actin is shown as a loading control and quantitative analysis of LC3-II is presented. b Cells were incubated in medium containing normal glucose (5.5 mmol/L) or 40 mmol/L HG with or without 100 nmol/L Rap for 48 h and were subjected to phase-contrast microscopy (magnification, ×200). c Cells were incubated in medium containing normal glucose (5.5 mmol/L) or 40 mmol/L HG with or without 100 nmol/L Rap for 48 h and were subjected to Annexin V and PI assays, and the percentage of apoptotic cells was quantified. d Cells were incubated in medium containing normal glucose (5.5 mmol/L) or 40 mmol/L HG with or without 4 μmol/L emodin and 100 nmol/L Rap for 48 h and were subjected to Western blot analysis of Caspase-3 and Cleaved caspase-3 levels. The level of β-actin is shown as a loading control and quantitative analysis of Cleaved caspase-3 is presented. #P < 0.01.

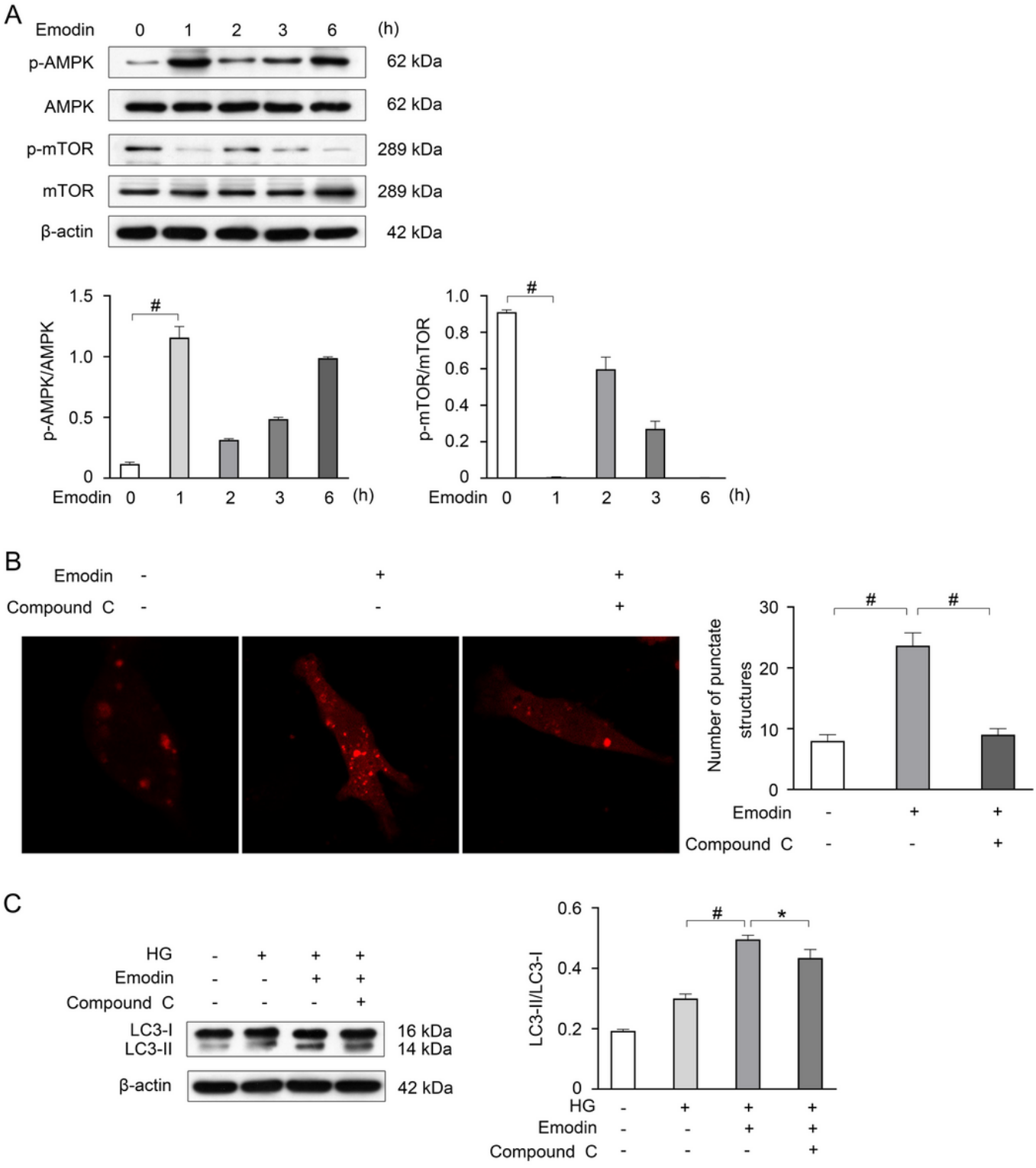

Figure 5

Emodin induces autophagy by regulating the AMPK/mTOR signaling pathways. a MPC5 cells were treated with 4 μmol/L emodin for 0–6 h and were subjected to Western blot analysis of p-AMPK, AMPK, p-mTOR and mTOR protein levels. The level of β-actin is shown as a loading control and quantitative analysis of p-AMPK, p-mTOR are presented. b Fluorescent microscopic analysis of MPC5 cells transfected with pmRFP fluorescent-tagged LC3 plasmid and exposed to 4 μmol/L emodin with or

without 10 μmol/L compound C for 1 h. The number of punctate structures was quantified. c Cells were incubated in medium containing normal glucose (5.5 mmol/L) or 40 mmol/L HG with or without 4 μmol/L emodin and 10 μmol/L compound C for 48 h and were subjected to Western blot analysis of LC3-I and LC3-II protein levels. The level of β-actin is shown as a loading control and quantitative analysis of LC3-II is presented. *P < 0.05, #P < 0.01.