Autophagy inhibits inflammation via down-regulation of p38 MAPK/mTOR signaling cascade in endothelial cells

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Abstract

Autophagy, an intracellular process of self-digestion, has been shown to modulate inflammatory responses. In the present study, we determined the effects of autophagy on inflammatory response induced by supernatant of psoriatic dermal mesenchymal stem cells (p-DMSCs). Human umbilical vein endothelial cells (HUVECs) were treated with supernatant of p-DMSCs cultures to induce inflammation and treated with rapamycin (RAPA) to induce autophagy. Expression levels of mRNA for inflammatory cytokines and BIRC2 were compared in HUVECs with vs. without induction of autophagy with rapamycin (RAPA) by PCR, while cell apoptosis was assessed by flow cytometry and caspase-3 activity assay kit. We found that induction of autophagy with RAPA decreased expression levels of IL6, IL8 and CCL20, in addition to reduction in inflammation-induced apoptosis in HUVECs; Expression levels of LC3, p62, p-p38 MAPK (Thr180/Tyr182), p-mTOR (Ser2445) and p-ULK1 (Ser555) proteins were measured by Western blotting. We found RAPA increased LC3 ，while decreasing p62 expression. Likewise, expression levels of p-p38 MAPK and p-mTOR proteins were markedly decreased by the treatment with RAPA; Finally, we evaluated thenitric oxide (NO) content, NO synthase (NOS) activity and cell angiogenesis. RAPA treatment increased the NO content and the NOS activity, and inhibited angiogenesis. Through the experimental results, we speculated that induced of autophagy can improve the function of endothelial cells in psoriasis, suggesting approaches to induce autophagy can be used to ameliorate psoriasis.

Introduction

Autophagy is an evolutionarily conserved catabolic process that degrades cytoplasmic materials and provides a substrate for energy metabolism during nutrient deficiency and metabolic stress in order to maintain cellular homeostasis and adapt to adverse environments (Jiao Liu et al, 2020; Younis Hazari et al, 2020; Beth Levine et al, 2008; Lorenzo Galluzzi et al, 2014; Romana T Netea-Maier et al, 2016). Alterations of autophagy are associated with a number of inflammatory diseases, including psoriasis (Dennis J Wu et al, 2017; Xue Mei Li et al, 2020). A large number of studies have shown that autophagy and autophagy-related proteins are involved in immune regulation, such as intracellular bacterial clearance, secretion of inflammatory cytokines, antigen presentation and lymphocyte development (Dennis J Wu et al, 2017; Yuan Cao et al, 2019). Autophagy is initiated by inducing autophagy genes for microtubule-associated protein light chain 3 (LC3), Beclin-1 and other autophagy related proteins, which all play an important role in the maintenance cell homeostasis under physiological and pathological conditions (Douglas R Green et al, 2011; Beth Levine et al, 2011; Qiuhong Zhang et al, 2013).

The regulation of autophagy is a very complex process. Mammalian target of rapamycin (mTOR), phospinositide 3-kinase (PI3K)/ Akt, MAPK and other pathways are considered as major regulatory pathways of autophagy and have been widely studied (Zhifen Yang et al, 2010; Zhifen Yang et al, 2010). Mammalian rapamycin mechanistic target is a typical inhibitor of autophagy, which is related to growth factor nutrient and energy signals. Rapamycin can inhibit mTOR complex 1 (mTORC1), which effectively inhibits autophagy by phosphorylating ULK1. In addition to regulation of autophagy, the mTORC1 signaling pathway also regulates various processes in innate immune cells through various mechanisms

such as metabolic protein translation and antigen presentation (Jung Hwa Ko et al, 2017). In addition, mitogen-activated protein kinase (MAPK) signaling pathway regulated cell growth and differentiation. MAPK pathway is also considered to be the main regulation pathway of autophagy (Xinbing Sui et al, 2014).

Psoriasis is a chronic, multifactorial, immune-mediated skin disease (Alan Menter, 2016). Psoriasis is considered as a systemic disease because psoriatic inflammation is involved both cutaneous and extracutaneous tissues (Zeinab Aryanian et al, 2021). Previous studies demonstrated that abnormal autophagy contributes dermal angiogenesis (Felix Locker et al, 2018), neovascularization, and extravasation of inflammatory cells into the lumen in psoriasis (K A Rubina et al, 2017). Since autophagy deficiency can induce the production of proinflammatory cytokines by increasing the expression of p62 (María-José Barrera et al, 2021), inflammation in psoriatic endothelial cells could also be linked to altered autophagy. Therefore, we studied here the regulatory role of autophagy in inflammation, apoptosis and endothelial cell function in vitro.

Materials And Methods

Materials

Materials and sources were as following: EBM-2 (Lonza, Germany,lot NO.: 9MB833), rapamycin (Solarbio, Beijing, Lot. No.: 1018N033),trypsin solution (Gibco Invitrogen, New York, USA, lot NO.: 1563418),eECL Western Blot Kit (CWBIO, Beijing, China, Lot NO.:20507), SB203580 (Med Chem Express, New Jersey, CAS No.: 152121-47-6), Chloroquine (CQ) (Med Chem Express, New Jersey, CAS No.: 54-05-7), BD matrigel (Corning, NewYork, USA, lot No.: 6172007), Annexin V-FITC/PI Apoptosis Assays Kit (KeyGenBio TECH, Nanjing, China, Cat. NO.: KGA107), capase-3 Kit (Beyotime, Shanghai, China, Lot NO.: 070320200803), NO Kit (JianCheng, Nanjing, China, NO.: A013-2-1), NOS Kit (JianCheng, Nanjing, China, NO.: A014-2-2), DAPI (Solarbio, China, lot-no.20170412). Antibodies against β-actin, LC3, p62 were obtained from Abcam (Cambridge, England). p38 MAPK (8690), p-p38 MAPK (Thr180/Tyr182; 4511), ULK1 (8054), p-ULK1 (Ser555, 5869), mTOR (2983) and p-mTOR (Ser2445; 5536) were obtained from Cell Signaling Technology (Bossdun, USA).

Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) and psoriatic dermal mesenchymal stem cells (p-DMSCs) were cultured as described previously (Ling Zhou et al, 2021). Supernatant of p-DMSCs culture was collected and stored in 4℃ refrigerators. HUVECs at about 70%-80% confluency were treated with p-DMSC supernatant for 4h (p-HUVEC). Prior to the treatment with p-DMSC supernatant, autophagy of HUVECs was induced by incubation of HUVECs with 200nM rapamycin (RAPA) in EBM-2 for 1h (R-p-HUVEC).

Quantitative RT-PCR (qRT-PCR)

Quantitative RT-PCR was used to assess the expression levels of IL6, IL8, CCL20 and BIRC2 in HUVECs with and without RAPA treatment, as described previously (Ruixia Hou et al, 2013). Total RNA was extract from control, p-HUVEC and R-p-HUVEC. RNA was reversely transcribed into cDNA. For the PCR assay, cDNA was mixed with QuantiTect SYBR Green PCR Master Mix, primers, and RNase-Free Water, and tested on Step One™. Primers information is shown in Table 1.

Table 1
Primers used for RT-PCR

Gene	Primers
β-actin	For: GCAAATGCTTCTAGGCGGACT
	Rev: CAATCTCATCTCGTTTTCTGCG
IL-6	For: GAC AAA GCC AGA GTC ATT CAG AG
	Rev: TTG GAT GGT CTT GGT CCT TAG CC
IL-8	For: TTGGCAGCCTTCCTGATTTC
	Rev: AACTTCTCCACAACCCTCTGCA
CCL20	For: ATTGTGCGTCTCCTCAGTAAAAA
	Rev: TGTGATGCTTAAACAAAGCAAAC
BIRC2	For: GAATCTGGTTTCAGCTAGTCTGG
	Rev: GGTGGGAGATAATGAATGTGCAA

Immunofluorescence

Cells on chamber slide were washed with PBS for 3 times, followed by fixation with 4% paraformaldehyde and permeation with 0.5%Triton x-100 for 20min at room temperature. After blocking with serum, cells were incubated with primary antibody LC3 (1:1000) overnight at 4℃. Afterward cells were incubation with secondary antibody for 1h. After DAPI staining, immunofluorescence staining was observed under an immunofluorescence microscope.

Western Blotting

Total protein was extracted from HUVECs for Western blot analysis. Cells were collected and lysed with ice-cold lysis buffer. Protein samples were bathed in metal bath for 10min at 95℃. LC3 was detected by traditional Western Blot. Briefly, a total of 20μg protein was loaded for Western blot assay. Electrophoresis was carried out using 12% separation glue and the transfer condition was 70V for 1.5h. Blotting was incubated overnight with LC3 rabbit primary antibody at 4℃, followed by washing with Tris-buffered saline containing 0.1% Tween for 3 times. The membrane was then incubated with a second antibody conjugated to horseradish peroxidase for 1h at room temperature. eECL Western Blotting reagent was

used to detect the labeled proteins. Imaging was performed using the Protein Simple Fluor Chem Q imaging system (Protein Simple, USA).

Protein Simple was used to detect the expression levels of p62, p38 mitogen-activated protein kinase (p38 MAPK), phosphorylated p38 MAPK, unc-51 like kinase 1 (ULK1), phosphorylated ULK1, mammalian target of rapamycin (mTOR) and phosphorylated mTOR. Protein samples and monoclonal antibodies against p62, p38 MAPK, p-p38 MAPK, ULK1, p-ULK1, mTOR, p-mTOR (antibody ratio 1:100) were added according to manufacturer's instructions, tested on WES system.

Apoptosis was detected by flow cytometry

After digestion and collection, cells were washed twice with PBS and centrifugated at 2000rpm for 5min. The cells were resuspended with 500μL Binding Buffer, followed by addition of 5μL Annexin V-FITC and 5μL Propidium Iodide. After incubation at room temperature for 5-15min in dark, apoptosis was detected by flow cytometry.

Measurement of caspase-3 activity

The cells were collected and the protein was extracted by adding 100μL lysate per $2*10^6$ cells. Protein concentrations were measured by Bradford's method and caspase-3 activity was detected with caspase-3 activity assay kit according to the manufacturer's instructions.

NO/NOS

Expression of NO and NOS activity were detected with respective kits. Assay was performed according to the manufacturer's protocol. The absorbance of OD value was measured at wavelength of 550nm with a microplate reader.

Angiogenesis experiment

The angiogenesis experiment was performed as described previously (Ling Zhou et al, 2018). Precooled tip was used to add BD matrigel glue to 96-well plate, 50μL/well. Afterward the cells were digested and inoculated with $1*10^4$cells/cm^2 for 24h, and then cultured at 37℃and 5% CO_2 for 6h. Under the microscope, five fields were randomly selected to count the numbers of junction and mesh. The data were expressed as percentages of control, and the control was set at 100%.

Statistic analysis

One way ANOVA with Tukey' s multiple comparisons was used to determine significant differences when three or more groups were compared, while an unpaired t test was used to determine significance between two groups. $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS.

Results

1. Induction of autophagy alleviates inflammation induced by supernatant of p-DMSCs

Because activation of autophagy can inhibit inflammation in croakers (Bo Yang et al, 2021), we first assessed here whether induction of autophagy can also inhibit inflammation in HUVECs. HUVEC inflammation was induced by incubation with supernatant of psoriatic mesenchymal stem cell culture (p-DMSC supernatant) for 4h. As seen in Fig. 1a, addition of p-DMSC supernatant to HUVEC culture significantly increased expression levels of mRNA for IL-6, IL-8 and CCL20, whereas induction of autophagy with RAPA lowered the expression levels of IL-6, IL-8 and CCL20 mRNA to the levels comparable to that of the controls. To ascertain whether RAPA induces autophagy, we measured expression levels of autophagy-associated biomarkers in the HUVEC cultures. As expected, RAPA treatment markedly increased the ratio of LC3I/LC3II protein (Fig. 1b, c), while decreasing p62 expression (Fig. 1d, e). In parallel, RAPA treatment increased fluorescence intensity of LC3 (Fig. 1f, g). The results show that RAPA induces autophagy, likely contributing to the alleviation of the p-DMSCs supernatant-induced inflammation in HUVECs.

2. Autophagy inhibits inflammation-induced apoptosis

Autophagy and apoptosis, different forms of cell death, interact with each other. Autophagy can antagonize apoptosis by promoting cell survival (Masakazu Hamada et al, 2017). We next determined whether autophagy can inhibit apoptosis of HUVECs. Flow cytometry showed a significant increase in p-HUVEC apoptosis (both early and late apoptosis) compared to the controls (Fig. 2a-d). Induction of autophagy with RAPA (R-p-HUVEC) significantly reduced apoptosis (both early and late apoptosis). Correspondingly, expression levels of BIRC2 mRNA, an inhibitor of apoptosis, were dramatically increased in R-p-HUVEC compared with p-HUVEC (Fig. 2e). In contrast, caspase-3 activity was significantly decreased following the treatment of p-HUVECs with RAPA (Fig. 2f), indicating an inhibition of apoptosis. These results indicate that autophagy inhibits inflammation-induced apoptosis in HUVECs.

3. Autophagy inhibits inflammation through the p38 MAPK/mTOR pathway

p38 MAPK pathway plays an important role in autophagy (Yannan Liu et al, 2019; Yingli He et al, 2018). ULK is the only core protein with serine/threonine kinase activity in autophagy signaling pathway. ULK1 complex acts as a bridge between upstream nutrient or energy receptor mTOR and downstream autophagosome in vivo. Phosphorylated ULK1 has long been considered a key regulator of autophagy (Chenyao Wang et al, 2018). To assess the involvement of p38 MAPK-mTOR-ULK1 signaling in the regulation of inflammation by autophagy, we measured expression levels of LC3, p62, p38 MAPK, p-p38 MAPK (Thr180/Tyr182), mTOR, p-mTOR (Ser2448), ULK1 and p-ULK1 (Ser555). p-HUVEC displayed significantly higher expression levels of LC3 (Fig. 3a, b) and p62 (Fig. 3c) compared with controls. RAPA treatment increased the ratio of LC3 /LC3I in R-p-HUVEC while decreasing p62 expression, indicating an induction of autophagy. Chloroquine (CQ) can inhibit autophagy by lysosomal acidification and subsequently blocks the fusion of the autophagosome with lysosome, leading to the accumulation of

autophagosome. Inhibition of autophagy with CQ increased both the ratio of LC3 Ⅱ / LC3Ⅰ and p62 expression (Fig. 3a-c), indicating autophagy initiation is normal, but autophagy flow is disrupted. Inhibition of p38 MAPK with SB203580 increased LC3 Ⅱ protein expression while decreasing p62 expression, indicating enhanced autophagy (Fig. 3a-c). In addition, the expression levels of both p-p38 MAPK and p-mTOR in R-p-HUVEC and SB-R-p-HUVEC were significantly decreased compared with p-HUVEC (Fig. 3d, f), and the expression of p-p38 MAPK in SB-R-p-HUVEC was significantly decreased compared with R-p-HUVEC (Fig. 3d), indicating that p38 MAPK is a negative regulator of autophagy. In contrast, inhibition of autophagy with CQ increased expression levels of p-mTOR in RAPA-treated p-HUVECs (Fig. 3f). On the other hand, p38 MAPK inhibitor increased the expression of p-ULK1 in RAPA-treated p-HUVEC while autophagy inhibitor, CQ, decreased p-ULK1 (Fig. 3e). The results show that RAPA-induced autophagy negatively regulates inflammation through the p38 MAPK/mTOR pathway.

4. Induction of autophagy improves the function of inflamed endothelial cells

Dysfunctions in psoriatic endothelial cells include increased pro-inflammatory response, decreased vasodilation, vasogenesis and thrombosis (Laura Mercurio et al, 2020). The decreased vasodilation is attributable to the reduced production of nitric oxide (NO) (Billie K Alba et al, 2018). Therefore, we detected NO content and NOS activity in HUVECs. As can be seen in Fig. 4a & b, both NO content and NOS activity declined significantly in p-HUVECs vs. normal controls. Induction of autophagy with RAPA significantly increased both NO content and NOS activity in p-HUVECs, while inhibition of autophagy reversed the effect of RAPA on both NO content and NOS activity, indicating that autophagy increases NO content and NOS activity in p-HUVECs.

Finally, we assessed whether the influence of autophagy on HUVEC function is reflected in angiogenesis, an abnormality in psoriasis. Our results showed that the numbers of both junction and mesh were increased and mean mesh areas were decreased in p-HUVECs in comparison to normal controls (Fig. 4c, d). Inhibition of autophagy overcame the effect of RAPA on the formations of junction and mesh, mean mesh areas in p-HUVECs. In contrast, neither the numbers of junction nor mesh or mean areas of mesh differed significantly in R-p-HUVECs treated with or without p38 MAPK inhibitor. Taken together, these results demonstrate that autophagy improves the function of inflamed endothelial cells via inhibition of p38 MAPK.

Discussion

The psoriasis-involved skin is characterized by increased blood vessels and angiogenesis, suggesting the pathogenic role of blood vessels and endothelial cells in psoriasis. So far, the studies on the role of endothelial cells in the pathogenesis of psoriasis are limited although regulation of $CARD14^+$ ECs in production of cytokines and chemokines (IL-8 and CXCL1 etc.) has been documented (Jamie L Harden et al, 2014). However, the pathomechanisms of endothelial cell inflammation in psoriasis have not been well defined. Since it is difficult to culture psoriatic endothelial cells from psoriatic skin, we established a psoriatic endothelial cell model by incubation of HUVECs with the culture supernatant of p-DMSCs. This

model exhibits some phenotypes of psoriatic endothelial cells such as increased expression levels of IL-6, IL-8 and CCL20. We show here that altered autophagy contributes, at least in part, to the development of inflammation in psoriatic endothelial cells.

Autophagy, also called programmed type II death, is a conserved degradation of the cells, removing unnecessary or dysfunctional components through a lysosome-dependent regulated mechanism, which is different from apoptotic programmed type death (Yufeng Xi et al, 2020; Dmitri V Krysko et al, 2008). Autophagy and inflammation are highly intertwined cellular processes (Beth Levine et al, 2011). Autophagy exerts anti-inflammatory property by regulating innate immune signaling pathway and inflammatory body activity (Vojo Deretic et al, 2013; James Harris et al, 2011). Moreover, autophagy can also be activated during the occurrence of inflammation. Autophagy not only affects the relief of infectious diseases and the pathological process of inflammatory diseases, but also can inhibit inflammatory response to the damages of non-infectious tissues (Vojo Deretic et al, 2018). Growing evidences indicate that autophagy dysfunction not only causes psoriasis, but also aggravates the inflammation in the pathogenesis of psoriasis. Autophagy-related proteins regulate multiple immune functions, including secretion of inflammatory cytokines, bacterial clearance and lymphocyte development. Because of the possible pathogenic role of autophagy in psoriasis, it could be a target in the treatment of psoriasis (Hongpeng Lv et al, 2021). Correspondingly, in the present study, we demonstrate induction of autophagy inhibits inflammation and apoptosis in HUVECs.

The mechanism by which autophagy inhibits inflammation are not clear. But evidence points to the involvement of p38 MAPK/mTOR signaling pathway. MAPK is an important cellular transduction pathway, regulating cell growth and differentiation. Recent studies have shown that p38 MAPK, an intracellular signal transduction molecule, regulates a variety of inflammatory responses, including the expression of pro-inflammatory cytokines, leukocyte adhesion and chemotaxis. MAPK signaling pathways are involved in the regulation of autophagy (Shu-Ting Pan et al, 2015). Autophagy is a catabolic pathway regulated by a complex signal network. p38 MAPK is a stress-activated protein kinase because it is frequently activated in response to inflammatory responses induced by various environmental stresses (e.g., REDOX stress, UV irradiation of cytokines, heat shock and osmotic shock), which is a key process in the host defense system. Moreover, p38 MAPK regulates cell cycle, promoting apoptosis, differentiation and senescence (Yan Zhao et al, 2020) and inhibits basic autophagy by blocking Atg9 (Jemma L Webber et al, 2010). Furthermore, ULK1 is a key upstream regulator of autophagy (Yingli He et al, 2018). p38α MAPK can directly phosphorylate ULK1 and inhibit ULK1 kinase activity, leading to destruction of ULK1 functional complex with ATG13, consequently resulting in reduced autophagy. In the present study, we showed that p38 MAPK was activated by inflammation, and induction of autophagy reduced the phosphorylation of p38 MAPK. Additionally, mTOR is a serine/threonine protein kinase and a key negative regulator of autophagy initiation (Andrea Williams et al, 2008). It phosphorylates an important autophagy protein ULK1 and inhibits its activity, thereby preventing the formation of ULK1-ATG13-FIP200 complex and inhibiting autophagy (Ian G Ganley et al, 2009). Finally, mTOR signaling pathway inhibits autophagy by phosphorylating the transcription factor EB and preventing its nuclear translocation from expressing autophagy genes (Rei Unno et al, 2020). Our

results showed that either induction of autophagy or inhibition of p38 MAPK lowered expression levels of p-mTOR. Thus, autophagy-induced inhibition of inflammation is in part via inhibition of p38 MAPK-mTOR-ULK1 signaling pathways.

Considering the correlation between NO/NOS and endothelial cell function, we assessed the expression levels of NO and the activity of NOS. NO, synthesized by NOS, has a variety of physiological and pathological functions. NO is essential for maintaining microvascular endothelial cell function and vascular homeostasis by inducing vasodilation and inhibiting platelet adhesion and aggregation (Hiroki Saito et al, 2018). Under inflammatory conditions, the physiological activity of eNOS may be impaired, leading to a so-called uncoupled state characterized by the production of superoxide $O2^-$ instead of NO (Susanne Karbach et al, 2014). Therefore, the bioavailability of NO in patients with psoriasis is reduced, leading to systemic microvascular dysfunction. Reduced bioavailability of nitric oxide impaired endothelium-dependent vasodilation (Susanne Karbach et al, 2014). The results of the present study showed that induction of autophagy increased NO expression and NOS activity, and stimulated endothelial angiogenesis. In agreement with our findings, other studies showed that autophagy stimulates No production (Cezar Rangel Pestana et al, 2015), but the specific mechanism remains to be elucidated.

Conclusion

In this study, we established a psoriatic endothelial cell model to investigate the effects of autophagy on inflammation and apoptosis of HUVEC. Induction of autophagy can inhibit inflammation and apoptosis, while improving endothelial cell functions in HUVECs treated with p-DMSC supernatant, mediated in part by p38 MAPK/mTOR signaling pathway. Induction of autophagy can improve the function of endothelial cells in psoriasis, potentiating the utility of approaches to enhance autophagy in the treatment of psoriasis.

Abbreviations

p-DMSCs
Psoriatic dermal mesenchymal stem cells
HUVECs
Human umbilical vein endothelial cells
LC3
Light chain 3
p38 MAPK
p38 Mitogen-activated protein kinase
ULK1
unc-51 like kinase 1, mTOR:mammalian target of rapamycin, RAPA:Rapamycin
CQ
Chloroquine

NO
Nitric oxide
EGM-2
Endothelial cell growth medium-2.

Declarations

Ethics approval and consent to participate

Ethical approval for the experiments was obtained from the Medical Ethics Committee of Taiyuan City Centre Hospital, and all subjects provided informed consent.

Consent for publication

Not applicable.

Availability of data

All data are available upon request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

LZ, JW and KZ designed the study and approved the final version. JL, XN, RH collected the clinical data. LZ and JL performed the experiments and analyzed the data. LZ and JW, HH and JL assembled and interpreted the data, wrote the manuscript. LZ and KZ are responsible for the integrity of this work and revised the manuscript. JL and JL assisted with cell culture, immunostaining and microscopy. LZ, JW and KZ take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Figures

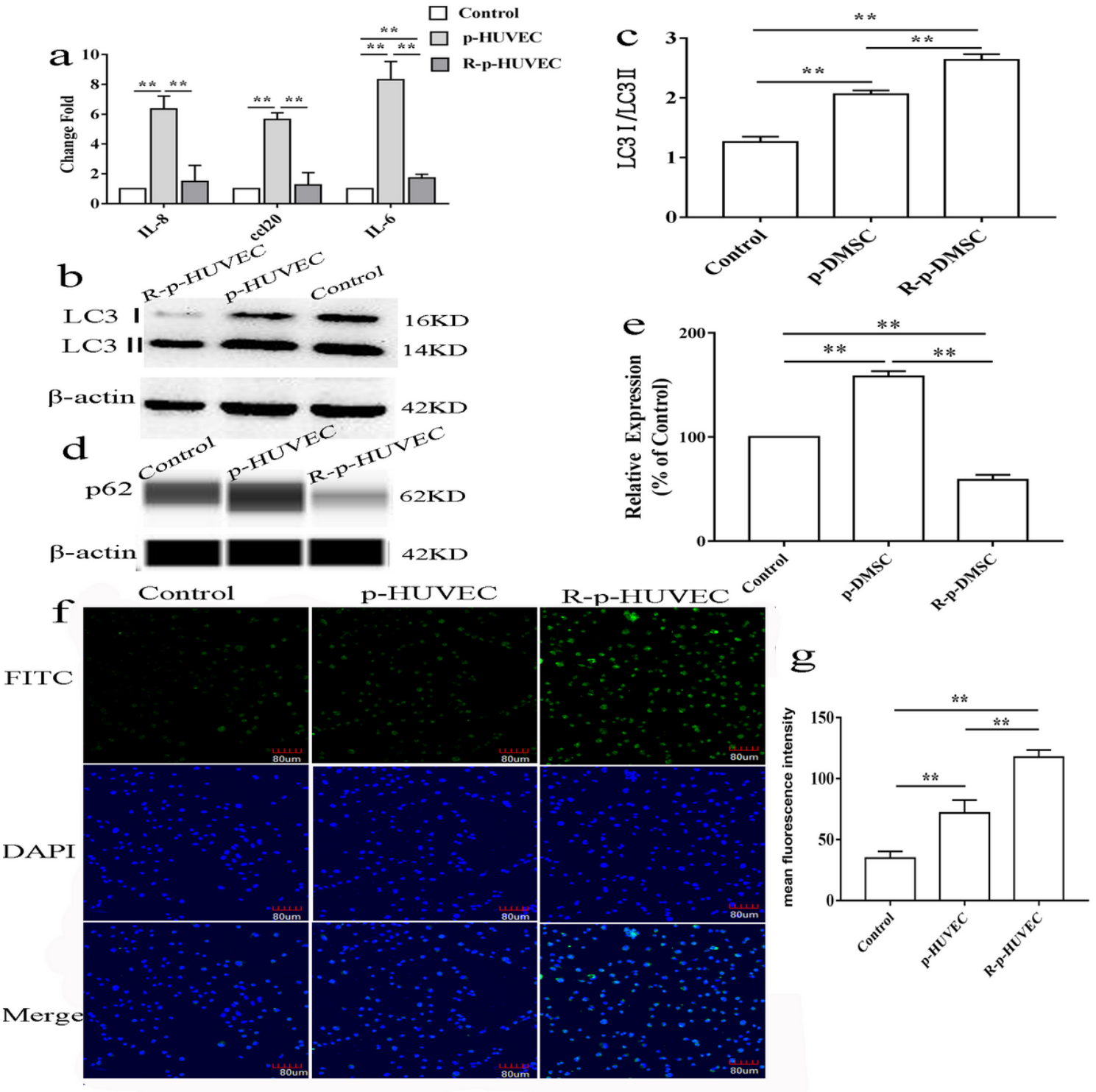

Figure 1

Induction of autophagy alleviates inflammationin HUVECs. Inflammation of HUVECs was induced by addition of p-DMSC supernatant to the cultures, while autophagy was induced by the treatment of HUVECs with Rapamycin. 1a. Expression levels of mRNA for IL6, IL8 and CCL20; 1b & c. Expression levels of LC3 protein and ratio of LC3I/LC3II; 1d & e. Expression levels of p62 protein; 1f & g. Expression of LC3 assessed by immunofluorescence. n=5, *p<0.05, **p<0.01.

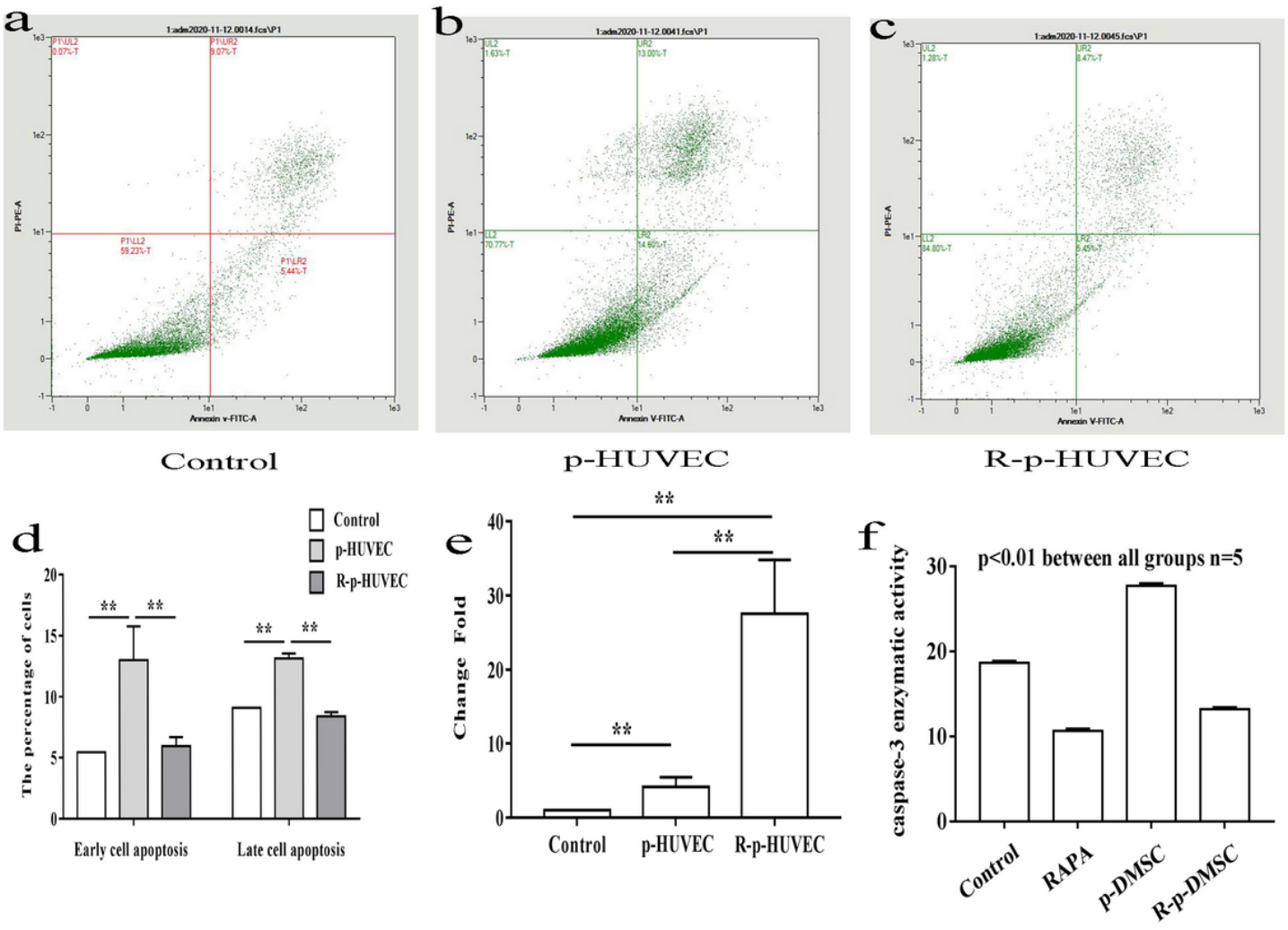

Figure 2

Induction of autophagy inhibits apoptosisin HUVECs. 2a-c. Cell apoptosis assessed by flow cytometry; 2d. Quantitative data of percentage of apoptotic cells; 2e. Expression levels of BIRC2 mRNA, and 2f. caspase-3 activity. n=5, *p<0.05, **p<0.01.

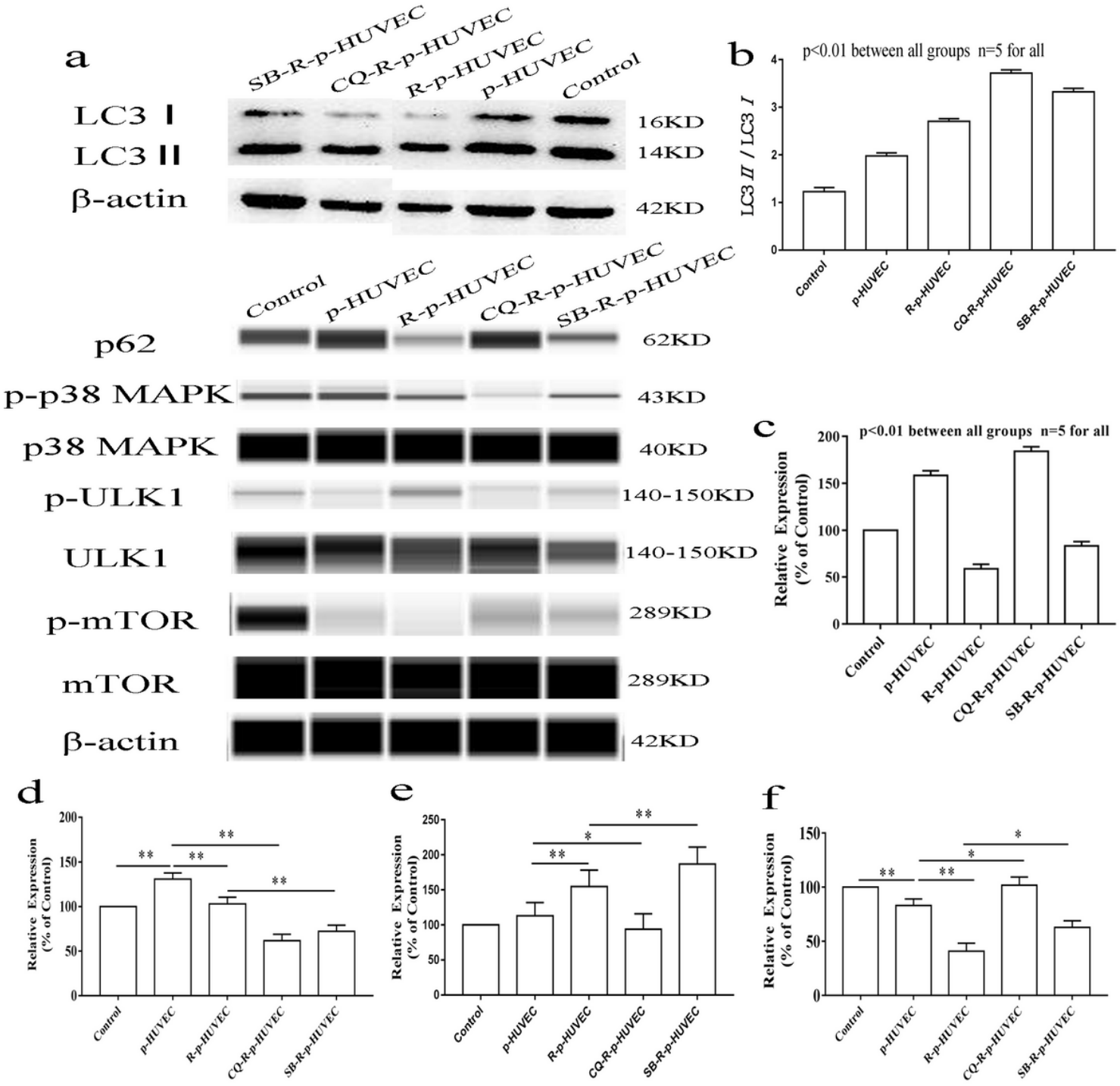

Figure 3

Autophagy-induced inhibition of inflammation is via p38 MAPK/mTOR signaling pathway.3a is the representative images of western blot; 3b & c are quantitative diagrams of expression levels of LC3 and p62; 3d, e & f are quantitative diagrams of expression levels of p-p38 MAPK, p-ULK1, and p-mTOR, respectively. n=5, *p<0.05, **p<0.01.

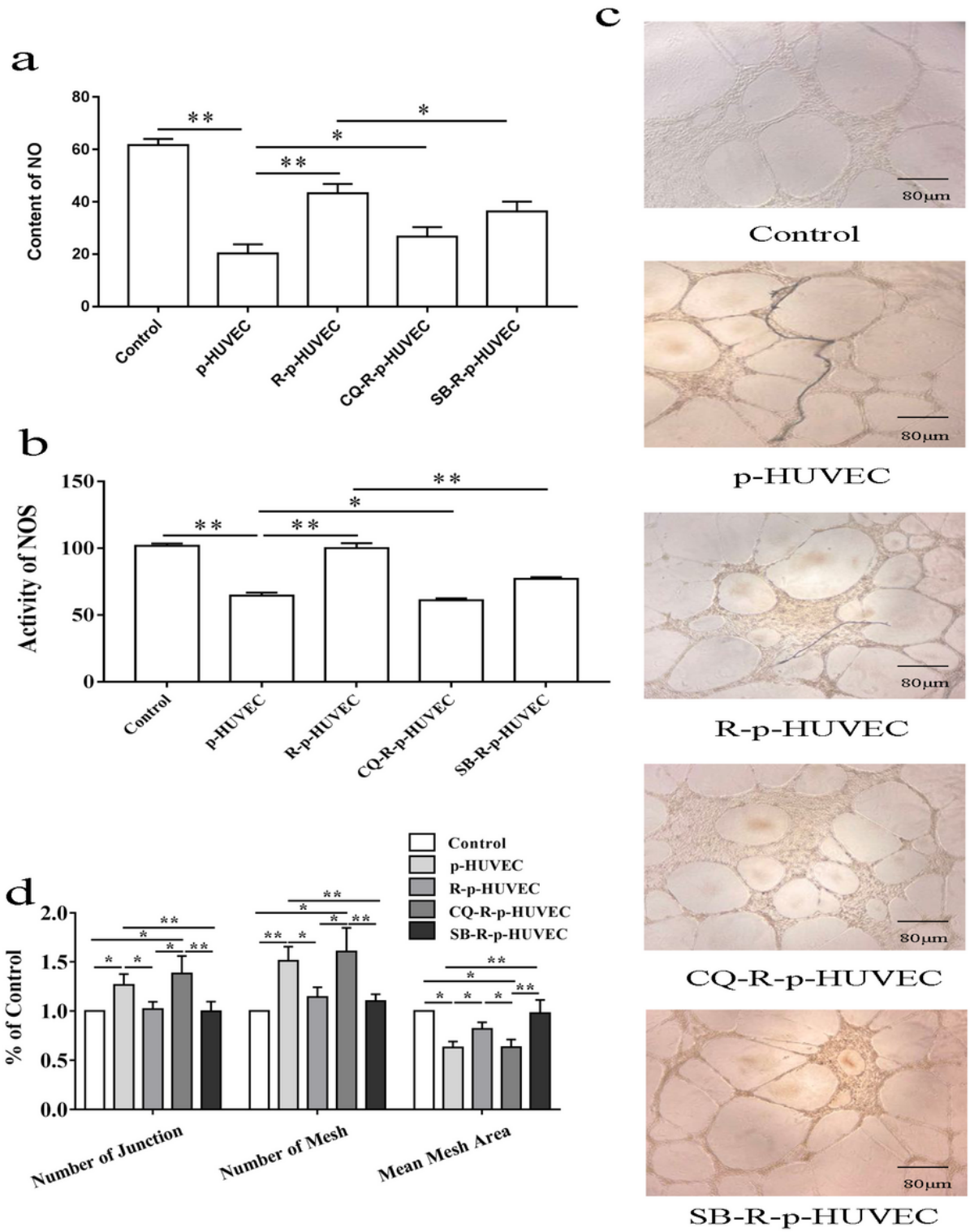

Figure 4

Autophagy improves endothelial cell function. 4a & b show NO levels and NOS activity, respectively; 4c and d display representative images of angiogenesis and quantitative diagrams of angiogenesis in HUVEC culture, respectively. n=5, *p<0.05, **p<0.01.