Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Resveratrol attenuates cigarette smoke induced endothelial apoptosis by activating Notch1 signaling mediated autophagy

Dandan Zong
Department of Respiratory and Critical Care Medicine, the Second Xiangya Hospital, Central South University
Xiang-ming Liu
the Second Xiangya Hospital, Central South University
Jin-hua Li
the Second Xiangya Hospital, Central South University
Ruo-yun Ouyang
the Second Xiangya Hospital, Central South University
Ying-jiao Long
the Second Xiangya Hospital, Central South University
Ping Chen (pingchen0731@csu.edu.cn)
https://orcid.org/0000-0001-6707-8636
Yan Chen
the Second Xiangya Hospital, Central South University

Research

Keywords: Resveratrol, Notch1 signaling, endothelial cells, apoptosis, autophagy

Posted Date: May 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-27268/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published on January 19th, 2021. See the published version at https://doi.org/10.1186/s12931-021-01620-3.

Abstract

Background Endothelial apoptosis contributes to the pathogenesis of chronic obstructive pulmonary disease (COPD). Our previous studies have validated Notch1 as an anti-apoptotic signaling in cigarette smoke (CS)-induced endothelial apoptosis. Resveratrol (RESV) is a naturally occurring polyphenol that exhibits an anti-apoptotic activity in endothelial cells that exposed to many kinds of destructive stimulus. However, the effects of resveratrol on Notch1 signaling in CS-induced endothelial apoptosis have not yet been fully elucidated. Therefore, the aim of this study was to examine whether RESV can protect endothelial cells from cigarette smoke induced apoptosis via regulating Notch1 signaling.

Methods HUVECs were pretreated with RESV for 2 h, followed by cotreatment with 2.5%CSE for 24h to explore the role of RESV in CSE induced endothelial apoptosis. 3-MA or rapamycin was used to alter autophagic levels. Lentivirus Notch1 intracellular domain (LV-N1ICD) or γ-secretase inhibitor (DAPT) were used to change Notch1 expression. The expression of Notch1, autophagic and apoptotic markers were examined by Western blot and the apoptosis rate was detected by Flow cytometry analysis.

Results Our results showed that activating autophagy reduced CSE-induced endothelial apoptosis, while blocking autophagy promoted cell apoptosis in HUVECs. RESV pretreatment attenuated the CSE-induced endothelial apoptosis and activated Notch1 signaling. RESV pretreatment also increased LC3b-II and Beclin1 production, decreased p62 and mTOR expression. 3-MA treatment inhibited autophagy and aggravated CSE induced apoptosis, while rapamycin promoted autophagy, led to a decrease in cell apoptosis. LV-N1ICD transfection upregulated autophagy and reduced apoptosis. However, this protective effect was abolished by 3-MA treatment. In cells treated with DAPT, autophagy was decreased, while apoptosis was increased. RESV partly rescued the DAPT induced apoptosis by activating Notch1 signaling.

Conclusion In HUVECs, RESV attenuates CSE induced endothelial apoptosis by inducing autophagy in a Notch1-dependent manner.

Background

Chronic obstructive pulmonary disease (COPD) as a common cause of disability and mortality is a global health threat. It has been currently estimated to be the third leading cause of death worldwide [1]. Cigarette smoke (CS) is well known to be a predominant risk factor for the development and progression of COPD. Increasing evidence demonstrated that the abnormal apoptosis of structural cells, such as alveolar epithelial cells and vascular endothelial cells, serves as a significant factor contributing to the destruction of pulmonary tissue in COPD [2]. Our previous studies confirmed that endothelial apoptosis plays an important role in the pathogenesis of emphysema and COPD in both animal models and human clinical studies [3–5]. Moreover, cigarette smoke extract (CSE) can induce endothelial apoptosis in dose- and time-dependent manner [6–10]. Thus, attenuating endothelial apoptosis is the key to reversing the progression of COPD.

Resveratrol (3, 4', 5-trihydroxystilbene; RESV), a natural polyphenol phytoalexin isolated from a variety of plant species, is a well-recognized anti-inflammatory, anti-oxidant, anti-aging and cancer chemopreventive agent. A growing body of evidence suggests that RESV may delay the onset of a number of diseases, including cardiovascular disease, diabetes, cancer and neurodegenerative diseases [11]. However, up to now, only a few studies explored the role of RESV in COPD. RESV was confirmed to exert anti-apoptotic effects, which protect the endothelial cells against the adverse effects of CS-induced oxidative stress [12]. Nevertheless, the underlying mechanisms remain uncharacterized. Autophagy is a fundamental intracellular process, which can degrade microorganisms (viruses, bacteria, fungi and protists/protozoa) and damaged organelles and proteins that cannot be degraded by the proteasome, providing raw materials for cell reconstruction, regeneration, and repair, thereby ensuring the metabolic balance of cells. Furthermore, RESV has been found to exert a protective effect via regulating autophagy [13]. However, no study has focused on the role of RESV-induced autophagy in CS-related endothelial apoptosis.

Notch1 is a transmembrane receptor that mediates intracellular signaling involved in cell differentiation, survival, and apoptosis. Work by our group and others has validated the Notch1 pathway as an anti-apoptotic pathway in CS-induced endothelial apoptosis [6, 7]. As reported, RESV has been suggested to induce functional Notch1 protein expression and activated the pathway by transcriptional regulation [14, 15]. Additionally, increased Notch1 activation T-regulatory cells was necessary for the autophagy induction and survival of cells in response to nutritional deprivation [16, 17]. Therefore, we hypothesized that the protective effect of RESV in response to CS-induced endothelial apoptosis occurs via Notch1-mediated autophagy. In the present study, we investigated whether RESV could regulate cell autophagy and apoptosis of human umbilical vein endothelial cells (HUVECs) that exposed to CSE, and whether RESV exerted a protective effect on CSE induced endothelial apoptosis via Notch1 signaling. Furthermore, we explored whether Notch1 signaling could function as an anti-apoptotic factor through regulating the activation of autophagy. This study will provide a better understanding of the pathogenesis of COPD.

Methods

Cell lines and cell culture

HUVECs were obtained from the American Type Cell Culture Collection (ATCC, lot no.: CRL-1730) and cultured in RPMI-1640 medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Gibco) in a humidified, 5% CO2 atmosphere at 37 °C. Cells were passaged at 80% confluence and grown to full confluence for the experiments. Each experiment was performed in triplicate and repeated independently at least three times.

Preparation of CSE

CSE was prepared as previously described [6]. Briefly, one nonfiltered Fu-Rong cigarette (tar: 12 mg/cigarette; nicotine: 1.1 mg; and carbon monoxide: 14 mg; China Tobacco Hunan Industrial.,

Changsha, China) was burned, and the smoke was passed through 25 ml of phosphate-buffered saline via a vacuum pump. This 100% CSE solution was adjusted to 7.2 ~ 7.4 and filtered through a 0.22-m membrane filter to remove large particles and bacteria before use. CSE was freshly prepared within the 30 min preceding each experiment.

Cell treatment

After serum starvation for 24 h, HUVEC medium was supplemented with 2.5% CSE for 24 h as described previously [10]. To investigate the role of RESV in CSE induced apoptosis, after serum starvation, HUVECs were pretreated with 40 μM RESV (R5010, Sigma-Aldrich Co., St Louis, MO, USA) for 2 h, followed by cotreatment with 2.5% CSE for 24 h. To examine the effects of autophagy on HUVEC apoptosis, the cells were cultured for 2 h in the presence or absence of the potential 5 mM of the autophagy inhibitor 3-methyladenine (3-MA) (A8353, APExBIO, Houston, TX, USA) or 50 nM of the autophagy inducer rapamycin (Rapa) (A8167, APExBIO, Houston, TX, USA) prior to CSE intervention. For Notch1 inhibition, HUVECs were treated with γ-secretase inhibitor DAPT (D5942, Sigma-Aldrich Co., St Louis, MO, USA) for 24 h.

Flow cytometry analysis

Apoptosis was assessed using an Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, USA). After treatment, cells were harvested and washed twice with ice-cold PBS and binding buffer and incubated with AnnexinV-FITC in dark for at least 15 min. Propidium iodide (PI) was added in the dark for 10 min at room temperature. Apoptosis was determined by flow cytometry (A00-1-1102, Beckman, USA). The degree of early apoptosis was determined as the percentage of cells positive for Annexin V and negative for PI by flow cytometry. Cells staining negative for Annexin V and PI were considered to be normal. Cells staining positive for Annexin V and negative for PI represented early apoptosis. Finally, cells staining positive for both Annexin V and PI were classified as late apoptosis.

Lentivirus Notch1 Preparation and Transfection

cDNA encoding a constitutively active form of Notch1 consisting of the intracellular domain (N1ICD, base pairs: 5,308-7,665; amino acids: 1,770-2,555) was synthesized and subcloned into the multicloning site of the lentiviral vector pNL-IRES2-EGFP to generate pNL-N1ICD-EGFP by the GeneChem Corporation (Shanghai, China). N1ICD and the vector lentivirus were transfected into HUVECs using Lipofectamine 3000 (ThermoFisher Scientific, USA) following the manufacturer's instructions. Cells of each group were collected 48-72 hours after transfection. The transfection efficiency of N1ICD was confirmed by immunoblotting.

Western blot analysis

Cells were harvested and digested in a lysis buffer. Total protein concentration was determined by the BCA protein assay kit. Proteins were separated by 10% SDS-PAGE and were transferred onto PVDF membranes, then incubated with antibodies to N1ICD (#4147, Cell Signaling Technology, Danvers, MA,

USA), cleaved caspase-3 (#9664, Cell Signaling Technology, Danvers, MA, USA), LC3b (14600-1-AP, Proteintech Group, Inc., Rosemont, IL, USA), Beclin1 (11306-1-AP, Proteintech Group, Inc., Rosemont, IL, USA), mammalian target of rapamycin (mTOR) (20657-1-AP, Proteintech Group, Inc., Rosemont, IL, USA) and P62/SQSTM1 (18420-1-AP, Proteintech Group, Inc., Rosemont, IL, USA). After washing, membranes were incubated with HRP-conjugated secondary antibody for 1 h. The bound complexes were detected using enhanced chemiluminescence detection system (Santa Cruz Biotechnology, Dallas, TX, USA). β-actin (600008–1, Proteintech Group, Inc., Rosemont, IL, USA) was probed to confirm equal protein loading and transfer.

Statistical analysis.

Quantitative data are presented as the means ± SD. Statistical analysis was performed using a software package (SPSS 21.0, SPSS Inc., Chicago, IL, USA). One-way ANOVA was used for multiple groups to analyze any differences between two groups. Tukey's test or Dunnett's T3 test was used for post hoc multiple comparisons according to the homogeneity test of variance. A value of $p < 0.05$ was considered to be statistically significant.

Results

Effect of autophagy on CSE-induced endothelial apoptosis

According to our previous studies, for a high early apoptosis rate and moderate late apoptosis or necrosis rate, a 24 h treatment with 2.5% CSE was selected [10]. For the measure of the autophagic and apoptotic effects of CSE exposure, we assessed the expression level of the Beclin1, LC3B-II, p62, mTOR, cleaved caspase 3 by Western blot and detected the apoptosis rate by flow cytometry. As shown in Fig. 1, CSE treated HUVECs exhibited a significant increase in the accumulation of LC3B-II, the expression of Beclin1 and cleaved caspase 3 proteins, as well as the apoptosis rate. The expression of p62 and mTOR significantly decreased after CSE stimulation. To explored the effects of autophagy on CSE induced apoptosis, we pretreated HUVECs with the mTOR inhibitor rapamycin and the specific PI3K inhibitor 3-MA to promote or inhibit autophagy. The results of LC3B-II, Beclin1, p62 and mTOR expression levels showed significant difference between groups with and without pretreatment of rapamycin and 3-MA. Meanwhile the pretreatment of rapamycin significantly reduced the cleaved caspase 3 levels and the apoptosis rate induced by CSE. Moreover, pretreated with 3-MA exerted an opposite effect. These data suggested that CSE induced apoptosis of HUVEC could be reversed partly by autophagy in HUVECs.

RESV stimulates autophagy and protects HUVECs from CSE-induced apoptosis

To examine the effect of RESV on cell apoptosis, the cells were pretreated with or without RESV prior to CSE. As shown in Fig. 2, the LC3B-II and Beclin1 level increased significantly, while the expression level of p62 and mTOR decreased significantly in the RESV and CSE cotreatment group compared to the CSE

group (P□0.05). In contrast, after combined RESV and CSE treatment, there was a significant decrease in the cleaved caspase 3 expression level and a reduction in HUVEC apoptosis rate. These results indicated that RESV may attenuate CSE-induced apoptosis in HUVECs through activating autophagy.

RESV activate Notch1 signaling

To elucidate the molecular mechanisms underlying the protective effect of RESV on endothelial apoptosis, we further analyzed the expression level of N1ICD in HUVECs using western blot analysis. We examined the expression of the active constituent of Notch1 (N1ICD) in HUVECs treated with CSE in the presence or absence of RESV. As shown in Fig. 3, the protein expression of N1ICD induced by RESV were analyzed by Western blot. The results showed a decrease in N1ICD expression in HUVECs that exposed to CSE. After cotreated with RESV and CSE, the expression level of N1ICD restored. These results suggest a potential role for Notch1 in the protective effect of RESV.

RESV induced autophagy and reduced apoptosis in HUVECs in a Notch1-dependent manner

We next examined whether Nocth1 is involved in the protective effect of resveratrol against CSE-induced endothelial apoptosis. Following the transfection and intervention of HUVECs, we evaluated changes in the expression level of autophagic and apoptotic markers. As expected, RESV reversed the decrease in N1ICD expression by DAPT in HUVECs. After pretreated with RESV, the inhibition of LC3B-II and Beclin1 by DAPT were restored, and the expression levels of p62 and mTOR decreased. In addition, pretreated with RESV yielded a protective effect from apoptosis. Figure 4 showed us that DAPT led to a significant induction of cleaved caspase3 levels and apoptosis rate. RESV rescued the increased apoptotic protein expression in HUVECs treated with DAPT, and resulted in a corresponding decrease in apoptosis rate. These results demonstrated that RESV protected HUVECs from apoptosis by activating Notch1 signaling-dependent autophagy pathway.

Perturbations of Notch1 or autophagy induced HUVECs apoptosis

In the previous study, we have confirmed a protective role of Notch1 in CSE-induced endothelial apoptosis [6]. Here, we assumed that Notch1 signaling might protect HUVECs from apoptosis via activating autophagy. To prove this hypothesis, we altered autophagy and Notch1 expression at the same time. We used N1ICD lentivirus (LV-N1ICD) and the vector lentivirus (LV-NC) to establish overexpression of Notch1 in HUVECs. Meanwhile, we interfered Notch1 expression by DAPT. As shown in Fig. 5, we found that LV-N1ICD effectively promoted the expression of N1ICD in HUVECs compared with vector control ($p < 0.05$), while DAPT treatment significantly suppressed the N1ICD expression levels. The expression of LC3B-II and Beclin1 were increased in cells transfected with LV-N1ICD, and further increased in cells following resveratrol treatment. Simultaneously, the expression of p62 and mTOR decreased. On the contrary, DAPT

had an opposite effect on the expression levels of autophagic markers. These results suggested a promoting role of Notch1 in autophagy. However, compared to those without 3-MA or rapamycin, no change in N1ICD protein expression was found in cells treated with them, suggesting Notch1 to be an upstream regulator of mTOR signaling. Then, we detected the apoptosis level by Western blot and flow cytometry. In accordance with our previous study, Notch1 overexpression reduced the apoptosis of HUVECs, while the inhibition of Nocth1 increased the cell apoptosis. 3-MA treatment blocked the protective effect of Notch1 on apoptosis, while rapamycin restored the cell apoptosis induced by DAPT. In general, these data indicated that Notch1 protect apoptosis via activating autophagy in HUVECs.

Discussion

In the present study, we provide evidence that RESV can suppress CSE- induced endothelial apoptosis via autophagy induction. RESV treatment also resulted in an increased expression of active Notch1 in HUVECs. DAPT significantly blocked Notch-1 activation and increased apoptotic protein expression, which can be partially reversed by RESV. Furthermore, the protective autophagy induced by RESV in HUVECs depends on a Notch1 signaling. Taken together, these data suggest that restoration of Notch1 signaling and induction of autophagy play critical roles in the protective effect of resveratrol on CSE-induced endothelial apoptosis.

Apoptosis of pulmonary structural cells including pulmonary endothelial cells is significantly elevated in human smokers with COPD [3, 9], and mice with emphysema caused by CS exposure [4, 5]. Our previous studies also demonstrated that CSE triggers apoptosis of endothelial cells in vitro [6–8, 10]. Consistent with previous studies, the present study further confirmed the promoting effect of CSE on the apoptosis of HUVECs. It has been shown that direct induction of pulmonary endothelial cell apoptosis using an endothelial cell-specific peptide results in emphysematous lung destruction. Furthermore, inhibition of endothelial apoptosis effectively prevented the emphysematous changes [4, 5]. These results highlight a crucial role of pulmonary endothelial cell apoptosis in the pathogenesis of CS related COPD. Therefore, preventing CS-induced pulmonary endothelial cell apoptosis and strengthening the pulmonary endothelial barrier may be an effective therapeutic strategy for COPD.

RESV is a polyphenol naturally found in a variety of plant species such as grapes, apples, berries, and peanuts. It is well known to have beneficial effects, including anti-inflammatory, anti-oxidative stress and anti-apoptosis [11, 18]. The anti-apoptotic effects of RESV have been widely investigated in multiple organs, including lungs. Ceramide was reported to be up-regulated in the lungs of COPD patients and CS exposed mice, and was shown to be associated with the increased apoptosis of bronchial epithelial cells [20]. RESV protects bronchial epithelial cells from CS-induced apoptosis in vitro and in mouse models through attenuating the accumulation of ceramide [21]. As a natural activator of SIRT1, RESV is capable of protecting bronchial epithelial cells against CSE-induced apoptosis through activating SIRT1 [22]. The results indicated the antiapoptotic function of RES in bronchial epithelial cells in the condition of CS exposure. However, few studies investigated whether it has a similar protective effect on CSE-induced endothelial cell apoptosis. In the present study, we have strong evidence that that resveratrol attenuates

CSE- induced HUVEC apoptosis. Pretreatment with RESV remarkably attenuated the expression of cleaved caspase-3, an indicator for cell apoptosis, in the presence of CSE. Moreover, its protective effect against HUVEC apoptosis under CSE is associated with autophagy. Autophagy is an essential intracellular process responsible for the selective degradation of pathogens, damaged organelles and proteins that cannot be degraded by the proteasome, to support protein and organellar homeostasis, as well as recycling of biomolecular resources, to maintain energy homeostasis and cell survival [23]. Emerging evidence indicates that RESV attenuates endothelial oxidative injury in HUVECs by inducing autophagy[13, 24]. Here, in the present study we found that blocking autophagy with 3-MA aggravated the apoptosis by upregulating caspase3 expression, while inducing autophagy with rapamycin relieved the apoptosis, indicating that autophagy confers the ability to protect endothelial cells from CSE- induced apoptosis. Moreover, we observed a promoting effect of RESV on autophagy by inhibiting mTOR signaling pathway. Cells treated with RESV showed a decrease in the expression of mTOR protein, resulting in an upregulation in Beclin1 and LC3b-II expression, with a decrease in p62 levels compared to those without RESV treatment. However, the exact underlying mechanism remains unclear and requires further investigation.

Nocth1 receptor protein is one of the four single-pass transmembrane receptors in Notch system, which controls cell proliferation, differentiation, and apoptosis. Notch signal activation involves the ligand-receptor interaction from the surface of two neighbouring cells, which triggers two successive proteolytic cleavages and release of the NICD from the full-length heteromeric receptor. Then, NICD travels to the nucleus where it interacts with the DNA-binding protein and converts it into a potent transcriptional activator [25]. Our previous studies have demonstrated that Notch-1 was downregulated in lung tissue of COPD compared to those of non-smokers [6]. Furthermore, overexpressing Notch-1 has been shown to alleviate CSE mediated endothelial apoptosis [6, 7]. Recently, several studies have shown that Notch1 is involved in the autophagic process [17, 26]. We speculate that autophagy may be related to the protective effect of Notch1 against endothelial apoptosis. We found that Notch1 expression was decreased after CSE exposure in consistent with the previous studies. Notch1 inhibition leads to an increase in the expression of mTOR and a decrease Beclin1 and LC3b-II expression accompanied with increased p62 levels, as well as an increase in endothelial apoptosis. Overexpression of Notch1 induced autophagy by inhibiting mTOR signaling, meanwhile attenuated the HUVECs apoptosis in endothelial cells. Furthermore, rapamycin reversed the cell apoptosis resulting from the inhibition of Notch1, while 3-MA blocked the anti-apoptotic effect of Notch1 signaling. These results suggested that Notch1 heavily inhibits cell apoptosis of HUVECs by promoting mTOR-mediated autophagy.

Recent years, RESV has received increasing attention for its role in regulating the Notch signaling. However, conflicting results of RESV effects on Notch1 signaling were reported that RESV can enhance [14, 27] or suppress [28] Notch1 in a cell type dependent manner. In this work, we examined what role RESV plays in HUVECs in the condition of CSE exposure. We observed that RESV treatment significantly restored the Notch1 protein levels suppressed by CSE. Furthermore, supplementation with RESV improves the cell apoptosis in HUVECs resulted from the inhibition of Notch1 signaling. Taken together, the results

from the present study indicated that RESV elicits its protective effect against CSE-induced apoptosis through regulating Notch1-mediated autophagy.

Conclusions

In summary, our study suggested that RESV pretreatment is associated with relieved CSE-induced endothelial apoptosis at least partly via promoting autophagy mediated by a Notch1-dependent mechanism. Our results provide insights into the possibility that Notch1 is targeted by RESV and suggest that the associated induced autophagy may have implications for improving CSE-induced endothelial apoptosis. These findings support the therapeutic potential of RESV for the prevention and treatment of CSE-related diseases, such as COPD.

Abbreviations

3-MA: 3-methyladenine; COPD: chronic obstructive pulmonary disease; CS: cigarette smoke; CSE: cigarette smoke extract; DAPT: γ-secretase inhibitor; HUVEC: human umbilical vein endothelial cell; mTOR: mammalian target of rapamycin; NICD: Notch intracellular domain; Rapa: rapamycin; RESV: resveratrol

Declarations

Acknowledgements

Not applicable

Funding

This study was supported by the National Natural Science Foundation of China (Grant No. 81900042), the Natural Science Foundation of Hunan Province (Grant No. 2018JJ3763) and the National Key Clinical Specialty Construction Projects of China (Grant No. 2012-650).

Authors' contributions

Every author contributed to the reviewing of the paper. ZDD and LXM performed the laboratory work, statistical analyses, and drafted the manuscript. LJH and LYJ performed part of the laboratory work and statistical analyses. OYRY supervised the study and helped to draft the manuscript. CY and CP directed and corrected this research as corresponding author. All authors read and approved the final manuscript.

Availability of data and materials

Essential datasets supporting the conclusion are included in this published article

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

References

1. Rennard SI, Drummond MB. Early chronic obstructive pulmonary disease: definition, assessment, and prevention. Lancet. 2015;385:1778–88.
2. Zeng H, Kong X, Peng H, Chen Y, Cai S, Luo H, et al. Apoptosis and Bcl-2 family proteins, taken to chronic obstructive pulmonary disease. Eur Rev Med Pharmacol Sci. 2012;16:711–27.
3. Peng H, Yang M, Chen ZY, Chen P, Guan CX, Xiang XD, et al. Expression and methylation of mitochondrial transcription factor a in chronic obstructive pulmonary disease patients with lung cancer. PLoS One. 2013; 8:e82739.
4. Zhang H, Chen P, Zeng H, Zhang Y, Peng H, Chen Y, et al. Protective effect of demethylation treatment on cigarette smoke extract-induced mouse emphysema model. J Pharmacol Sci. 2013; 123:159 – 66.
5. Zeng H, Shi Z, Kong X, Chen Y, Zhang H, Peng H, et al. Involvement of B-cell CLL/lymphoma 2 promoter methylation in cigarette smoke extract-induced emphysema. Exp Biol Med (Maywood). 2016; 241:808 – 16.
6. Zong D, Li J, Cai S, He S, Liu Q, Jiang J, et al. Notch1 regulates endothelial apoptosis via the ERK pathway in chronic obstructive pulmonary disease. Am J Physiol Cell Physiol. 2018;315::C330-C40.
7. Long YJ, Liu XP, Chen SS, Zong DD, Chen Y, Chen P. miR-34a is involved in CSE-induced apoptosis of human pulmonary microvascular endothelial cells by targeting Notch-1 receptor protein. Respir Res. 2018;19:21.
8. Shi Z, Chen Y, Pei Y, Long Y, Liu C, Cao J, et al. The role of cyclooxygenase-2 in the protection against apoptosis in vascular endothelial cells induced by cigarette smoking. J Thorac Dis. 2017;9:30–41.
9. Peng H, Guo T, Chen Z, Zhang H, Cai S, Yang M, et al. Hypermethylation of mitochondrial transcription factor A induced by cigarette smoke is associated with chronic obstructive pulmonary disease. Exp Lung Res. 2019; 45:101 – 11.
10. Yang M, Chen P, Peng H, Zhang H, Chen Y, Cai S, et al. Cigarette smoke extract induces aberrant cytochrome-c oxidase subunit II methylation and apoptosis in human umbilical vascular endothelial cells. Am J Physiol Cell Physiol. 2015;308:C378-84.
11. Xia N, Daiber A, Forstermann U, Li H. Antioxidant effects of resveratrol in the cardiovascular system. Br J Pharmacol. 2017;174:1633–46.

12. Csiszar A, Labinskyy N, Podlutsky A, Kaminski PM, Wolin MS, Zhang C, et al. Vasoprotective effects of resveratrol and SIRT1: attenuation of cigarette smoke-induced oxidative stress and proinflammatory phenotypic alterations. Am J Physiol Heart Circ Physiol. 2008;294::H2721-35.
13. Du L, Chen E, Wu T, Ruan Y, Wu S. Resveratrol attenuates hydrogen peroxide-induced aging through upregulation of autophagy in human umbilical vein endothelial cells. Drug Des Devel Ther. 2019; 13:747 – 55.
14. LaFoya B, Munroe JA, Albig AR. A comparison of resveratrol and other polyphenolic compounds on Notch activation and endothelial cell activity. PLoS One. 2019;14:e0210607.
15. Yu XM, Jaskula-Sztul R, Ahmed K, Harrison AD, Kunnimalaiyaan M, Chen H. Resveratrol induces differentiation markers expression in anaplastic thyroid carcinoma via activation of Notch1 signaling and suppresses cell growth. Mol Cancer Ther. 2013;12::1276–87.
16. Marcel N, Sarin A. Notch1 regulated autophagy controls survival and suppressor activity of activated murine T-regulatory cells. Elife. 2016; 5.
17. Sarin A, Marcel N. The NOTCH1-autophagy interaction: Regulating self-eating for survival. Autophagy. 2017; 13:446–47.
18. Lu T, Zhou D, Gao P, Si L, Xu Q. Resveratrol attenuates high glucose-induced endothelial cell apoptosis via mediation of store-operated calcium entry. Mol Cell Biochem. 2018;442:73–80.
19. Song C, Luo B, Gong L. Resveratrol reduces the apoptosis induced by cigarette smoke extract by upregulating MFN2. PLoS One. 2017;12:e0175009.
20. Filosto S, Castillo S, Danielson A, Franzi L, Khan E, Kenyon N, et al. Neutral sphingomyelinase 2: a novel target in cigarette smoke-induced apoptosis and lung injury. Am J Respir Cell Mol Biol. 2011; 44:350 – 60.
21. Xuan LL, Shi J, Yao CS, Bai JY, Qu F, Zhang JL, et al. Vam3, a resveratrol dimer, inhibits cigarette smoke-induced cell apoptosis in lungs by improving mitochondrial function. Acta Pharmacol Sin. 2014; 35:779 – 91.
22. Zhang L, Guo X, Xie W, Li Y, Ma M, Yuan T, et al. Resveratrol exerts an anti-apoptotic effect on human bronchial epithelial cells undergoing cigarette smoke exposure. Mol Med Rep. 2015;11:1752–8.
23. Nakahira K, Pabon Porras MA, Choi AM. Autophagy in Pulmonary Diseases. Am J Respir Crit Care Med. 2016; 194:1196–207.
24. Zhou X, Yang J, Zhou M, Zhang Y, Liu Y, Hou P, et al. Resveratrol attenuates endothelial oxidative injury by inducing autophagy via the activation of transcription factor EB. Nutr Metab (Lond). 2019; 16:42.
25. Zong D, Ouyang R, Li J, Chen Y, Chen P. Notch signaling in lung diseases: focus on Notch1 and Notch3. Ther Adv Respir Dis. 2016;10:468–84.
26. Xu Y, Li Y, Liu X, Pan Y, Sun Z, Xue Y, et al. SPIONs enhances IL-10-producing macrophages to relieve sepsis via Cav1-Notch1/HES1-mediated autophagy. Int J Nanomedicine. 2019;14::6779–97.

27. Ji Y, Ke Y, Gao S. Intermittent activation of notch signaling promotes bone formation. Am J Transl Res. 2017;9:2933–44.

28. Zhong LX, Li H, Wu ML, Liu XY, Zhong MJ, Chen XY, et al. Inhibition of STAT3 signaling as critical molecular event in resveratrol-suppressed ovarian cancer cells. J Ovarian Res. 2015;8:25.

Figures

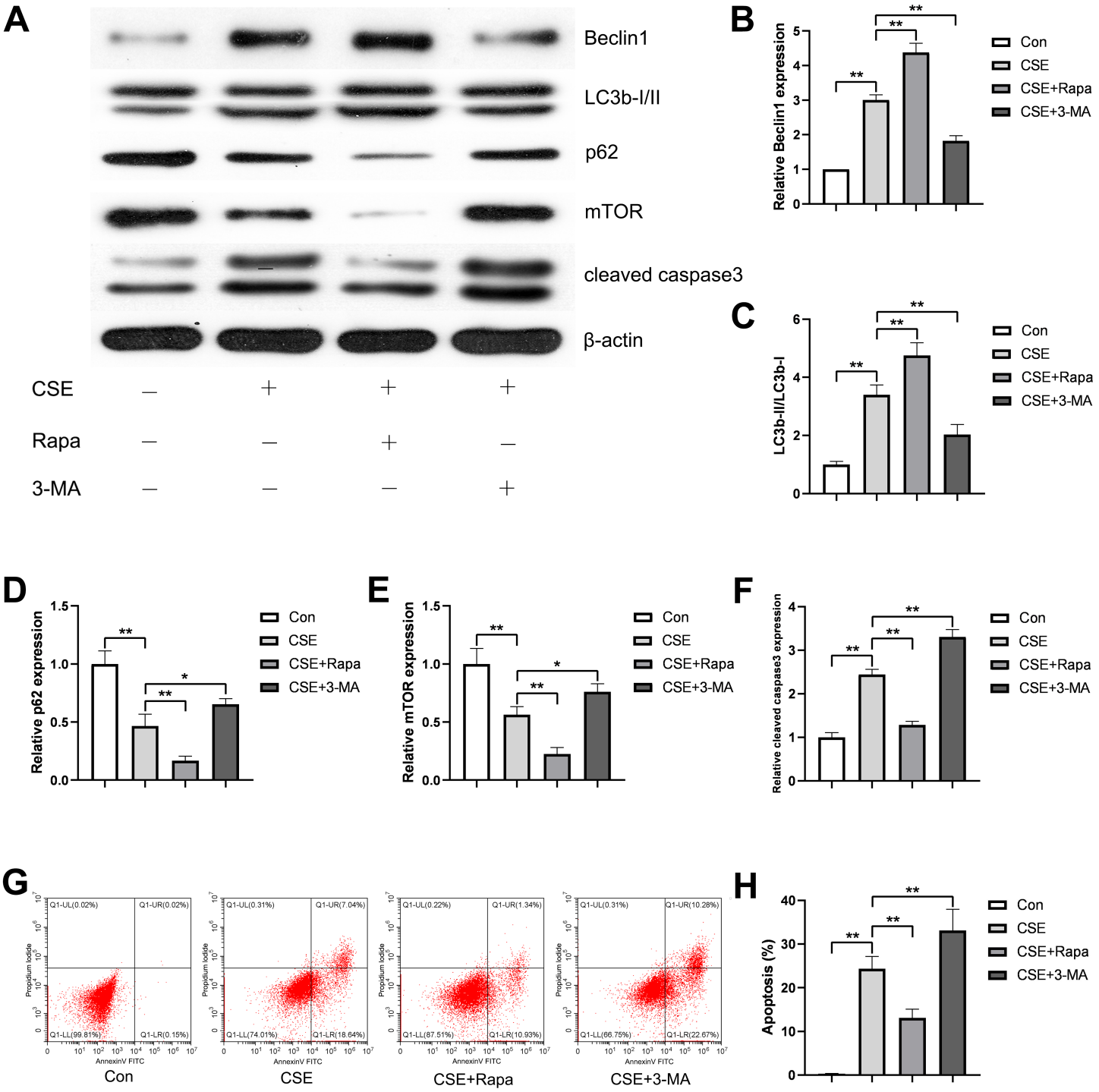

Figure 1

Effect of autophagy on CSE-induced endothelial apoptosis in HUVECs. HUVECs were incubated with 2.5% CSE for 24 h. To examine the effects of autophagy on HUVEC apoptosis, the cells were cultured for 2 h in the presence or absence of 5 mM 3-MA or 50 nM Rapa prior to CSE intervention. Autophagy related proteins (Beclin1, LC3b-I/II, p62, mTOR) and apoptotic protein (cleaved caspase3) were detected by Western blot. β-Actin was used as loading control (a). Bar charts show the quantification of Beclin1, LC3B-II/LC3b-I, P62, mTOR and cleaved caspase3. Values are presented as means ± SD (b, c, d, e, f). Cell apoptosis was detected by flow cytometry (g) and statistical analysis was calculated using One-way ANOVA (h). *P < 0.05, **P < 0.01 compared between the marked groups.

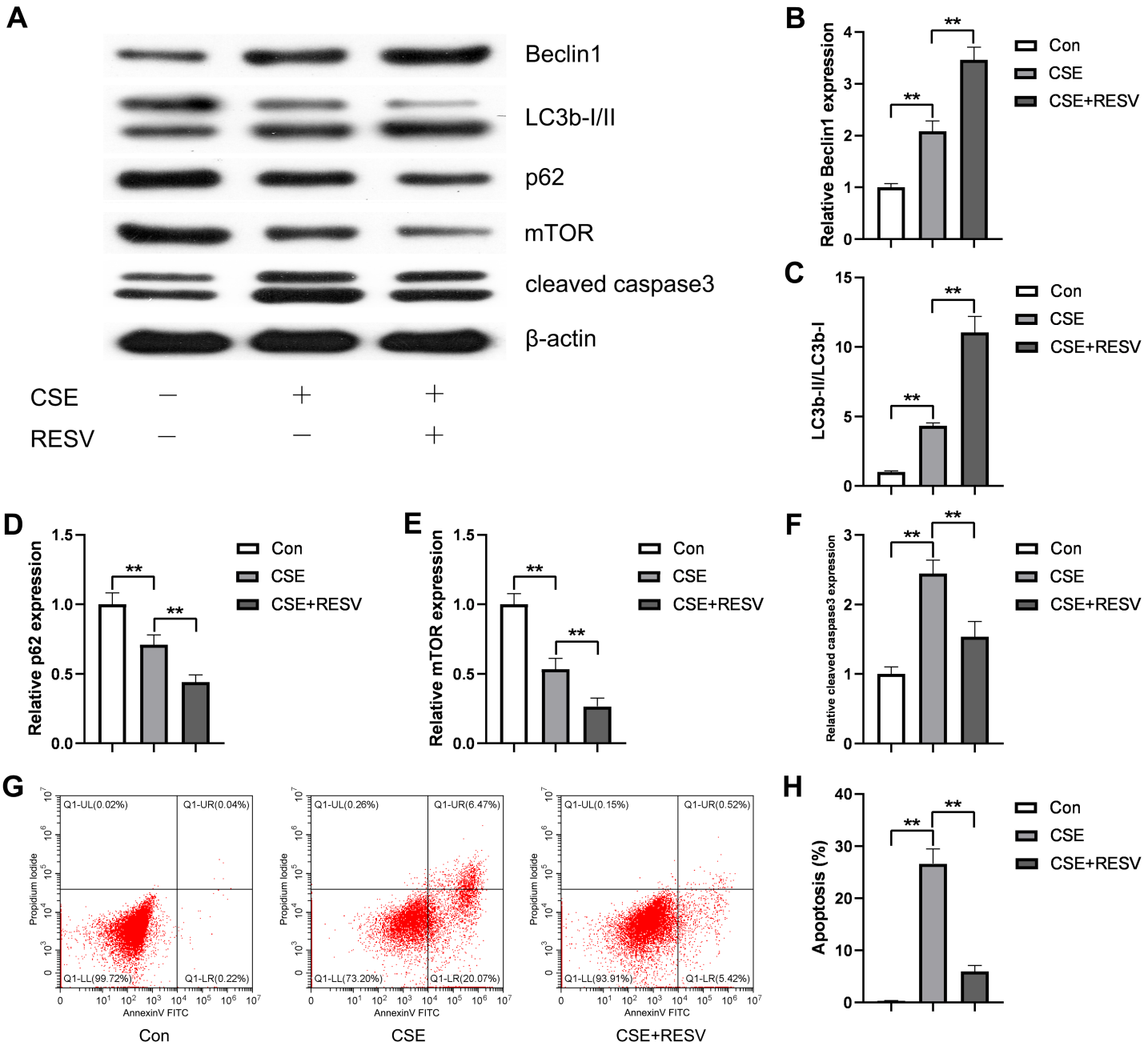

Figure 2

RESV attenuated apoptosis and induced autophagy in CSE treated HUVECs. To investigate the role of RESV in CSE induced apoptosis, HUVECs were pretreated with 40 μM RESV for 2 h, followed by cotreatment with 2.5% CSE for 24 h. Autophagy related proteins (Beclin1, LC3b-I/II, p62, mTOR) and apoptotic protein (cleaved caspase3) were detected by Western blot. β-Actin was used as loading control (a). Bar charts show the quantification of Beclin1, LC3B-II/LC3b-I, P62, mTOR and cleaved caspase3. Values are presented as means ± SD (b, c, d, e, f). Cell apoptosis was detected by flow cytometry (g). Statistical analysis was calculated (h). *P < 0.05, **P < 0.01 compared between the marked groups.

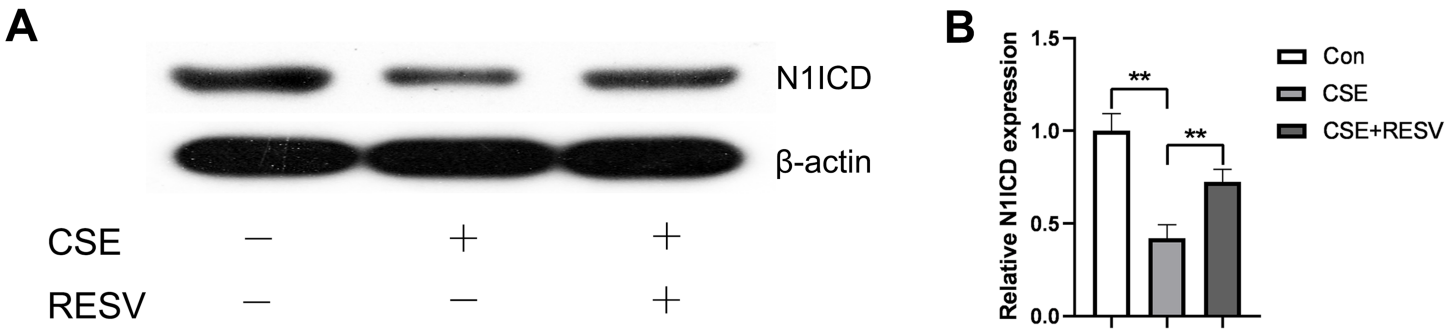

Figure 3

RESV restored the N1ICD expression. N1CID protein level was detected by Western blot. β-Actin was used as loading control (a). Bar charts show the quantification of N1ICD. Values are presented as means ± SD (b). *P < 0.05, **P < 0.01 compared between the marked groups.

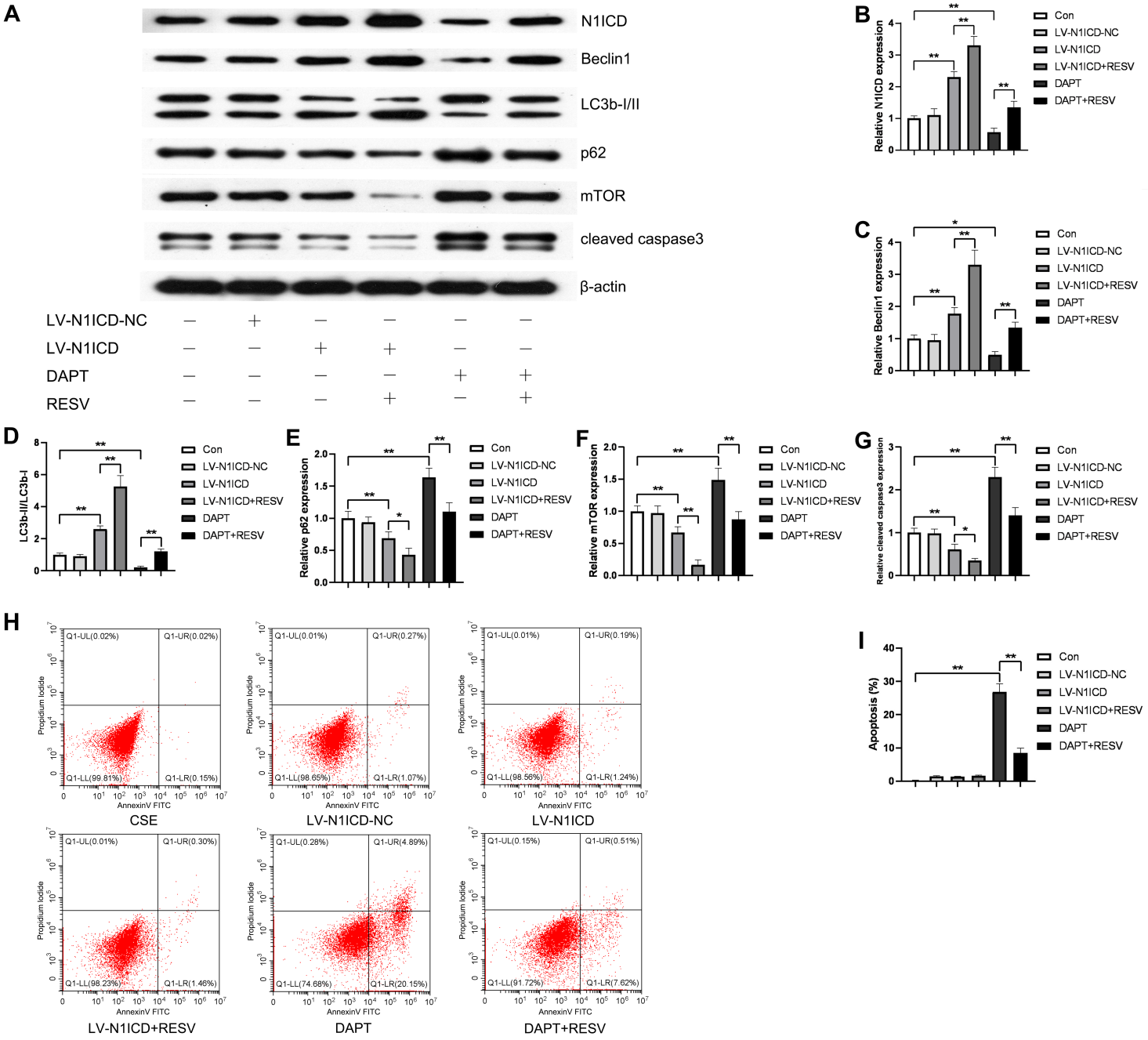

Figure 4

RESV stimulated autophagy and alleviated apoptosis through activating Notch1 signaling in CSE-treated HUVECs. N1ICD was overexpressed by LV-N1ICD transfection or suppressed by DAPT in HUVECs as described in the Methods section. N1CID protein, autophagy related proteins (Beclin1, LC3b-I/II, p62, mTOR) and apoptotic protein (cleaved caspase3) were detected by Western blot. β-Actin was used as loading control (a). Bar charts show the quantification of N1ICD, Beclin1, LC3B-II/LC3b-I, P62, mTOR and cleaved caspase3. Values are presented as means ± SD (b, c, d, e, f, g). Cell apoptosis was detected by flow cytometry (h). Statistical analysis was calculated (i). *P < 0.05, **P < 0.01 compared between the marked groups.

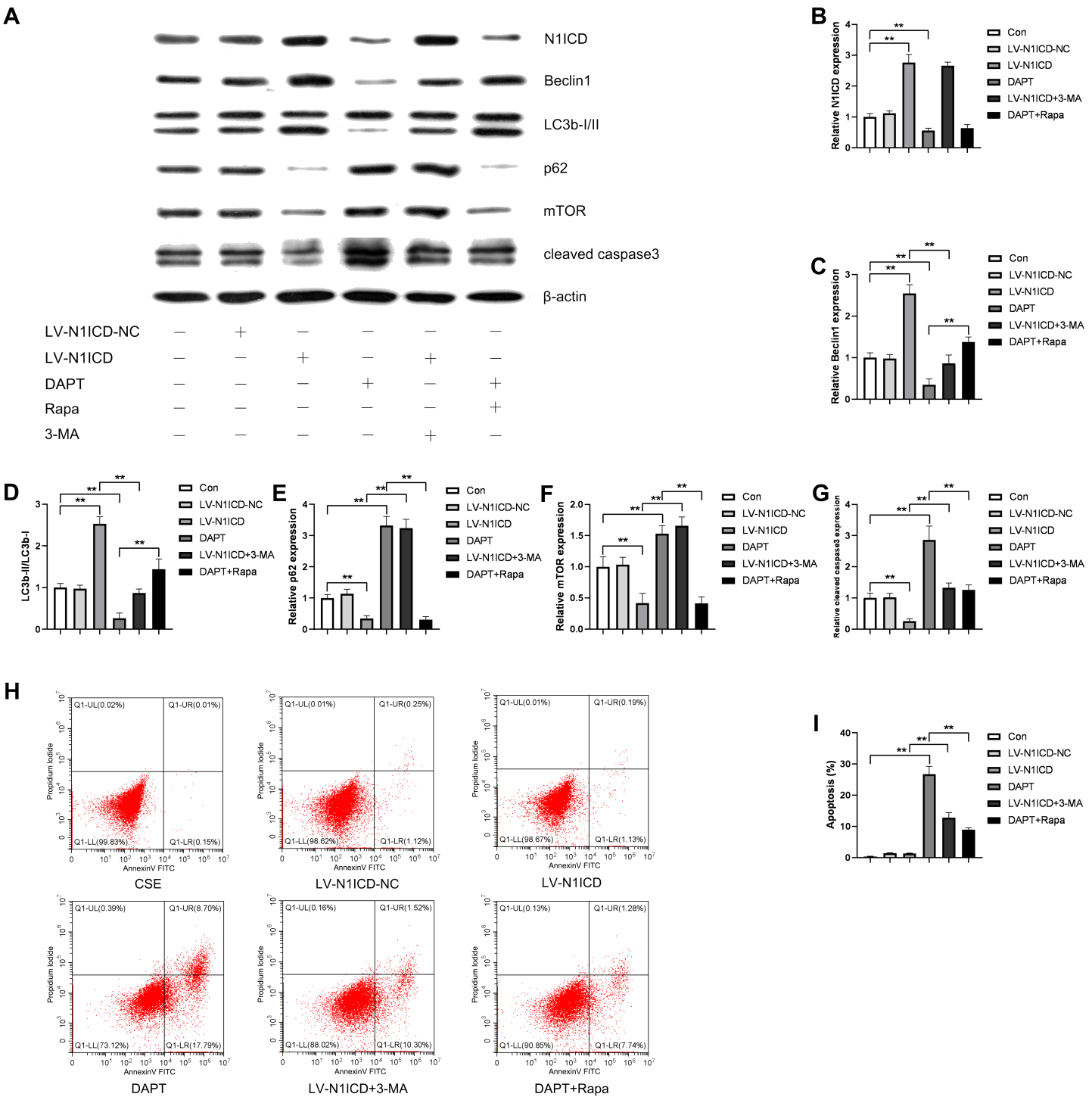

Figure 5

Notch1 protect HUVECs from CSE-induced apoptosis via activating autophagy. N1CID protein, autophagy related proteins (Beclin1, LC3b-I/II, p62, mTOR) and apoptotic protein (cleaved caspase3) were detected by Western blot. β-Actin was used as loading control (a). Bar charts show the quantification of N1ICD, Beclin1, LC3B-II/LC3b-I, P62, mTOR and cleaved caspase3. Values are presented as means ± SD (b, c, d, e, f, g). Cell apoptosis was detected by flow cytometry (h). Statistical analysis was calculated (i). *P < 0.05, **P < 0.01 compared between the marked groups.