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Original Article

Autophagy modulates the function of human umbilical vein endothelial cells exposed to hydrogen peroxide via Wnt/β-catenin signaling pathways

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Abstract: Background: Oxidative stress (OS) contributes to many negative effects, producing many intermediate products that mediate cell injuries, as observed in atherosclerosis (AS). Moreover, OS promotes endothelial-to-mesenchymal transition (EndMT). Autophagy and Wnt signaling pathways participate in the regulation of EndMT. Therefore, the current study examined whether autophagy and Wnt/β-catenin signaling pathways interact in human umbilical vein endothelial cells (HUVECs) under oxidative stress conditions. Methods: Expression levels of EndMT, Wnt/β-catenin signaling pathways, and autophagy markers in each group were measured via immunofluorescence and Western blotting. Evaluating the function of HUVECs exposed to H_2O_2, comprehensively, flow cytometry was used to measure apoptosis. Results: Results demonstrated that activating autophagy with Rap significantly prevented H_2O_2-stimulated HUVECs EndMT by downregulating α-smooth muscle actin (α-SMA) and upregulating CD31. It also showed a significant reduction in cell apoptosis ratios. Interestingly, activation of Wnt/β-catenin signaling pathways had similar effects to autophagy on cell apoptosis ratios. Activating Wnt/β-catenin signaling pathways with BIO could enhance levels of EndMT in HUVECs exposed to hydrogen peroxide. Enhancement of Wnt/β-catenin signaling pathways to EndMT could be attenuated by upregulation of autophagy. The promotion of Wnt/β-catenin signaling pathways to apoptosis could be attenuated by activating autophagy. Autophagy modulates the function of HUVECs exposed to oxidative stress partly via interaction with Wnt/β-catenin signaling pathways. Conclusion: Wnt/β-catenin signaling pathways and autophagy may be used to prevent the development of atherosclerosis, as EndMT contributes to the pathobiological process of atherosclerosis.

Keywords: Oxidative-stress, autophagy, Wnt/β-catenin pathways, EndMT, apoptosis

Introduction

Vascular endothelial cells have proliferation, adherence, angiogenesis, and secretory abilities. Many physiological and pathological processes involve autophagy [1]. Similarly, multiple pathophysiological processes are targets of Wnt/β-catenin or Wnt canonical pathways. Accumulating evidence has indicated that Wnt/β-catenin pathways control proliferation, senescence, and apoptosis in cells [2-4]. Moreover, activation of Wnt/β-catenin pathways could enhance EndMT [5]. Autophagy negatively regulates Wnt/β-catenin signaling pathways by promoting disheveled degradation [6, 7]. Cells exposed to H_2O_2 induce OS by increasing reactive oxygen species (ROS) and lipid peroxidation. Breaking these oxidant/antioxidant mechanisms leads to a state of oxidative stress.

Autophagy is a general term for the processes in which cytoplasmic materials, including organelles, reach lysosomes for degradation, achieving the metabolic requirement of the cell itself and the renewal of some organelles. At present, only microtubule-associated protein light chain3 (LC3) is known certainly to exist in autophagosomes. Therefore, many peers have

used this protein as a marker for autophagosomes. In addition, p62 directly binds to LC3 and is efficiently degraded by autophagy. Thus, total cellular expression levels of p62 inversely correlate with autophagic activity [8].

Previous studies have found that, in undergoing certain conditions, endothelial cells lose their specific markers and express mesenchymal cell products, such as α-smooth muscle actin (α-SMA) and type I collagen, called EndMT [9]. EndMT-derived cells enhanced migration and invasion capabilities, suggesting that the essential function of endothelial cells is changed. This has been found in the pathophysiological processes of many diseases. EndMT drives atherosclerosis progression, playing an important role during cardiac-cushion formation [10-15].

Wnt, β-catenin, APC, Axin, and serine/threonine kinase (GSK-3β) are downstream components of Wnt-mediated signaling pathways. Therefore, they have been used as markers of activation or inhibition levels of Wnt-mediated signaling pathways.

A previous work demonstrated that autophagy prevents human cardiac microvascular endothelial cells from undergoing EndMT [16, 17]. Canonical Wnt signaling activity is a characteristic property of EndMT [18]. Autophagy and Wnt/β-catenin pathways participate in the process of proliferation, apoptosis, and survival in HUVECs exposure to high glucose, hypoxia, or oxidative-stress. The current study was designed to investigate the cross talk of autophagy and Wnt/β-catenin signaling pathways that modulate the function of HUVECs in H_2O_2 condition.

Materials and methods

Materials

HUVECs isolated from human umbilical cords were obtained from the Department of Obstetrics and Gynecology, the First Affiliated Hospital of NanChang University. Endothelial Cell Basal Medium-2 (EBM-2) was obtained from Lonza (Walkersvlle, MD, USA). Moreover, 3-methyladenine (3-MA) and rapamycin (Rap) were purchased from Sigma-Aldrich (St. Louis, MO, USA). BIO-acetoxime (BIO) and XAV939 were purchased from Selleck (USA). Anti-actin, anti-CD31, anti-survivin, anti-LC3, anti-Beclin-1, anti-P62, and horseradish peroxidase-linked secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA). Anti-α-SMA, anti-FSP-1, anti-Wnt3a, and anti-β-catenin were purchased from Abcam (USA). CY3-co-njugatedgoat anti-mouse IgG and FITC-conjugated goat anti-rabbit IgG were purchased from Aspen (Wuhan, China). Annexin V-FITC apoptosis detection kit (Key GEN Bio TECH, Nanjing, China), SOD test kit, MDA test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and BCA Protein Assay Kit (Beyotime, Beijing, China) were purchased from the above companies. RIPA lysis buffer, PVDF or NC membranes, 5% non-fat milk, 4% paraformaldehyde, Triton-X 100, 2% BSA, and DAPI were purchased from Solarbio (Beijing, China). The protease inhibitor cocktail was purchased from Roche (Mannheim, Germany). Proteins were detected using the chemiluminescent kit purchased from Trans Gen Biotech (Beijing, China).

Isolation, identification, and culturing of HUVECs

Umbilical cords were collected in sterile normal saline solution supplemented with 100 U/mL of penicillin and 100 lg/mL of streptomycin. They were processed on a super clean bench within 2 hours. Next, 1 cm long pieces of cord were attached at both ends. Any damaged areas were resected with a scalpel. Upon measurement, the length of the remaining intact cord was typically 10-20 cm. One of the free ends of the umbilical vein lumen attached to a 2-cm long syringe needle. The tip was polished and clamped with tissue forceps. These syringe needles served as 'inlets' and 'outlets' to the umbilical vein lumen. Warm sterile normal saline solution was then injected 3 times to remove the remaining blood out of the umbilical vein lumen. The other end of the cord was clamped and type-2 collagenase at 37°C was injected to the lumen of the vein for a 10-minute incubation period. This was to digest the umbilical cord and separate HUVECs. During incubation, the cord was gently massaged. Importantly, during the process the cord was kept moist. After incubation, the non-needle end of the cord was unclamped. Type-2 collagenase and the separated HUVECs were collected in a 50 mL sterile centrifugal tube. This tube was loaded with 2 mL of fetal calf serum to

neutralize type-2 collagenase. Afterward, the vein was perfused with 30 mL of phosphate buffered saline (PBS) to recover remaining cells. Cells with the type-2 collagenase and PBS were centrifuged at 1,500 rpm for 10 minutes [19]. The supernatants were discarded and the pellets were carefully re-suspended in warm endothelial growth medium. All remains of the umbilical cords were treated as biohazards, according to institutional rules.

HUVECs were transferred to culture flasks coated with a thin layer of gelatin. These were left for at least 1 hour in the cell incubator. Coating solution was added to the culture flasks (125 ll/cm^2). Before cell seeding, the solution was removed. No washing steps were required. Cells were incubated under normal conditions. After 4 hours, the medium was refreshed. Traces of blood and cell debris were removed. HUVECs (passage 0) were left to grow for 2-3 days in the primary culture, changing the medium every other day. The cells were sub-cultured once they covered 85-90% of the culture plates. HUVECs (passage 2-5) were used in this study.

The cells displayed cobblestone-shaped and non-granular cytoplasm, available for further experiments. Healthy cells doubled in 2-3 days. Immunofluorescence confirmed that cells overexpressing CD31 (>98%) were high purity HUVECs, suitable for future experimentation.

HUVECs were divided into 8 groups for treatment, including the blank group, H_2O_2 (200 μM) induced group, H_2O_2 + Rap (100 nm) group, H_2O_2 + 3MA (5 mm) group, H_2O_2 + BIO (1 μM) group, H_2O_2 + XAV939 (1 μM) group, H_2O_2 + Rap + BIO group, and H_2O_2 + Rap + XAV939 group.

Spectrophotometry

Detection of intracellular superoxide dismutase (SOD) activity by spectrophotometry: After the culture medium was discarded, the HUVECs were digested with 0.25% trypsin. One minute later, the culture medium was added to stop the effects of pancreatin. After centrifugation of the cell suspension, the supernatant was abandoned and 50 μL cell lysate was added in each well. After gently pipetting to break up the cells, 40 μl lysate was taken. More medium was added to the samples and control wells, for a final volume of 220 μl. Next, 20 μl double-distilled water was added to control wells 1 and 3, respectively. After adding 200 μl of WST working fluid into the wells and 20 μl dilution buffer to control wells 2 and 3, 20 μl enzyme was added to the sample well and control hole 1, respectively. Incubation was conducted at 37°C for 20 minutes. The process was recorded with an enzyme scale at 450 nm for a total of 3 times.

Determination of malondialdehyde (MDA) content in cells by spectrophotometry: After the culture medium was discarded, the HUVECs were digested with 0.25% trypsin. One minute later, the culture medium was added to stop the effects of trypsin. After centrifugation of the cell suspension, the supernatant was abandoned. Next, 50 μl cell lysate was added to each well. After repeatedly pipetting the cells, 0.1 mL lysate was loaded to the MDA working fluid. After mixing and heating in a boiling water bath for 40 minutes, the lysate was cooled to room temperature. It was then centrifuged at 3,000 rpm for 10 minutes and measured at 535 nm with an enzyme labelling instrument.

Flow cytometry analysis

Fluorescein AnnexinV-FITC/PI double labeling was performed with the AnnexinV-FITC apoptosis detection kit, measuring apoptosis levels of HUVECs cells. They were seeded in 12-well plates and treated, as mentioned above. The cells were cultured under normal condition for 24 hours before they were stained with AnnexinV-FITC and PI, according to the kit manual. Stained cells were measured with flow cytometry (BD FACSCanto II; San Jose, CA).

Western blot analysis

All operations were performed on ice. The cells were homogenized in RIPA lysis buffer that contained a protease inhibitor cocktail. After the mixture was centrifuged at 12,000 g for 10 minutes at 4°C, the supernatant was collected. Protein concentrations were measured using a BCA Protein Assay kit, according to manufacturer protocol. Next, 25 μg protein was separated by 12% or 10% SDS-PAGE, then transferred onto PVDF membranes. The membranes were blocked with 5% non-fat milk for 1 hour, then incubated with primary antibodies (dilution rates: mouse anti-CD31 1:1000, rabbit anti-α-SMA 1:5000, rabbit anti-FSP1 1:2000, rabbit anti-LC3 1:1000, rabbit anti-beclin1

1:1000, rabbit anti-p62 1:1000, mouse anti-Wnt3a 1:1000, mouse anti-β-catenin 1:1000) at 4°C overnight. Proteins were detected using chemiluminescence kits.

Immunofluorescence staining

Endothelial cells were seeded in 24-well culture plates. After treatment, the cells were fixed in 4% paraformaldehyde for 15 minutes. They were then permeabilized with PBS containing with 0.1% Trition-X100. Next, 2% BSA was used to block it for 1 hour at room temperature, before further incubating with primary antibodies, monoclonal mouse anti-CD31 antibodies, monoclonal rabbit anti-α-SMA antibodies, or monoclonal mouse anti-LC3A/B antibodies at 4°C overnight. Afterward, the cells were mixed with secondary antibodies in a dark room. Cy3 red-conjugated goat anti-mouse IgG and FITC green-conjugated goat anti-rabbit IgG were mixed for 1 hour at room temperature. They were then incubated with 100 ul DAPI for another 15 minutes. The cells were visualized using confocal microscopy (Nikon, Tokyo, Japan). All images were processed using Photoshop CS 8.0 software.

Statistical analysis

GraphPad prism 7.0 Software was used to analyze all data. Quantitative data are presented as mean ± SD of three independent experiments. Student's *t*-tests were applied to compare differences between two groups. Means of multiple groups were compared using one-way ANOVA. $P<0.05$ indicates statistical significance.

Results

Oxidative stress promotes EndMT and apoptosis in HUVECs

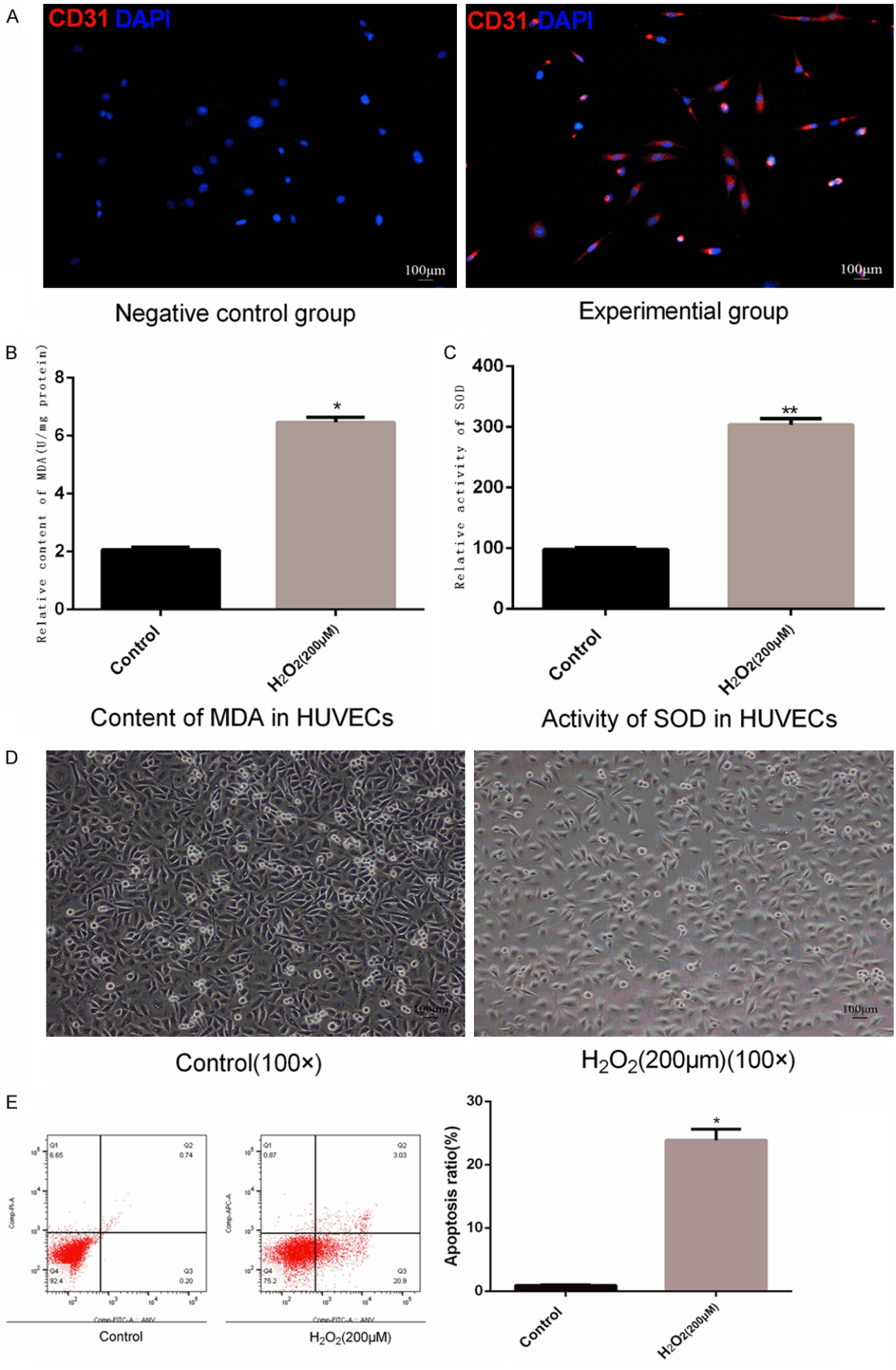
Immunofluorescence analysis confirmed that the isolated HUVECs overexpressed CD31 (**Figure 1A**), investigating the potential effects of hydrogen peroxide (H_2O_2) in HUVECs. After exposure to H_2O_2 (200 μM) for five days, the activity of intracellular SOD and MDA content in cells showed that H_2O_2 (200 μM) induction of group simulation of OS conditions was successful (**Figure 1B**, **1C**). Additionally, H_2O_2 (200 μM) simulation of OS conditions caused a change in morphology, with elongated spindle-shaped structure HUVECs observed (**Figure 1D**). Next, flow cytometry analysis was conducted to assess apoptosis in cells. Results showed that HUVECs exposed to H_2O_2 (200 μM) caused a decrease in cell number and an increase in cell apoptosis (**Figure 1E**). Showing changes in morphology in HUVECs exposed to H_2O_2, double immunofluorescence staining showed that H_2O_2-induced HUVECs lost proteins associated with endothelial cells (CD31), but expressed proteins associated with fibroblasts (α-SMA) (**Figure 1F**). In line with double immunofluorescence staining results, related protein expression levels of EndMT, examined by Western blotting, presented the same trend. Expression blots are presented in (**Figure 1G**). Present results suggest that H_2O_2-induced EndMT and apoptosis but inhibited proliferation in HUVECs. See (**Figure 1A-G**).

Oxidative stress activates expression of Wnt/β-catenin signaling pathways and promotes autophagy

The intracellular environment has effects on levels of autophagy and the activity of various signaling pathways. This study examined the related proteins of autophagy and Wnt/β-catenin signaling pathways. Results suggest that expression of P62 and Beclin-1 in the HUVECs group was downregulated, while LC3-II/LC3-I, Wnt3a, β-catenin, and survivin was upregulated (**Figure 2A**, **2B**), compared with the control group. Results showed that OS conditions could promote expression of autophagy related proteins in HUVECs. However, they could also activate Wnt/β-catenin signaling pathways, making expression of related proteins more obvious than expression in the control group.

Modulating levels of autophagic flux and Wnt/β-catenin signaling pathways can regulate EndMT and apoptosis in HUVECs

To further investigate the effects of autophagy and Wnt/β-catenin signaling pathways on EndMT and cell apoptosis of HUVECs under H_2O_2 conditions, this study treated induced-HUVECs with Rap, 3MA, BIO, and XAV939 from the third day for 2 days. Double immunofluorescence staining assays confirmed that CD31 was upregulated and α-SMA was downregulated in the Rap and XAV939 group, compared with the H_2O_2-induced group (**Figure 3A**). In con-


A
CD31 DAPI
100μm
Negative control group
CD31 DAPI
100μm
Experimental group
B
Relative content of MDA(U/mg protein)
8
6
4
2
0
*
Control
H2O2(200μM)
Content of MDA in HUVECs
C
Relative activity of SOD
400
300
200
100
0
**
Control
H2O2(200μM)
Activity of SOD in HUVECs
D
100μm
Control(100×)
100μm
H2O2(200μm)(100×)
E
Q1 6.65
Q2 0.74
Q4 92.4
Q3 0.20
Comp-PI-A
Comp-FITC-A :: ANV
Control
Q1 0.87
Q2 3.03
Q4 75.2
Q3 20.9
Comp-APC-A
Comp-FITC-A :: ANV
H2O2(200μM)
Apoptosis ratio(%)
30
20
10
0
*
Control
H2O2(200μM)

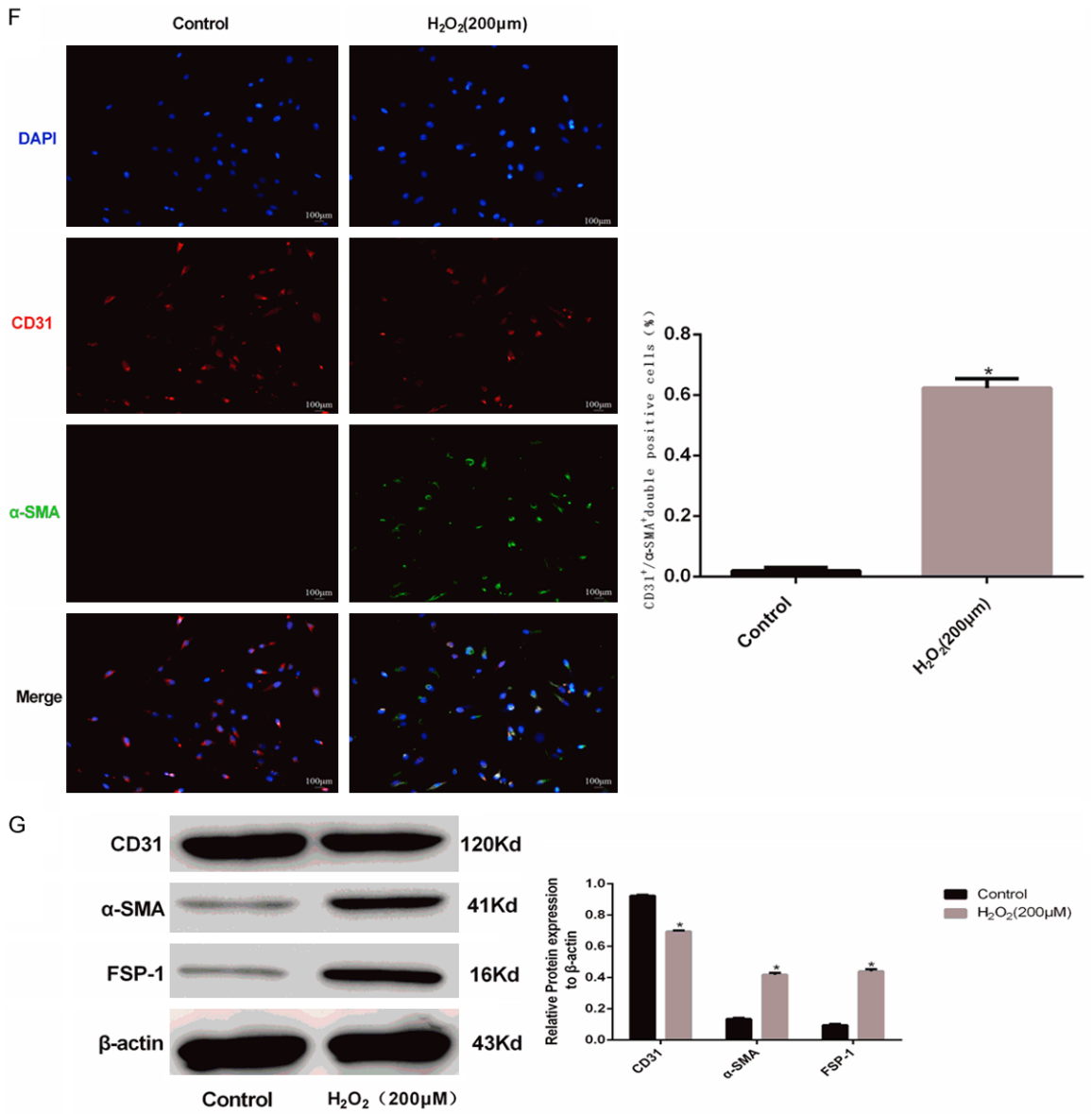


Figure 1. Effects of H_2O_2 (200 μM) on the function of HUVECs. A. Immunofluorescence staining with antibodies to CD31 (red). Nuclei were counterstained with DAPI (blue). Scale bars: 100 μm; B. Content of MDA in cells was detected by Spectrophotometry (*P<0.05 vs. control group); C. Concentrations of SOD in cells were detected by spectrophotometry (**P<0.05 vs. control group, n=7); D. Morphological changes in HUVECs are shown by representative phase-contrast light microscopy images (original magnification: ×100); E. Apoptotic cells were measured by annexin V-FITC/PI double staining (*P<0.05 vs. Control group, n=7); F. Double immunofluorescence staining: CD31 (red) and α-SMA (green). Counterstained nuclei with DAPI (blue). Scale bars: 100 μm; G. Western blot analysis of CD31, FSP1, and α-SMA in HUVECs from each group. β-actin was used as the loading control (n=7).

trast, upregulation of Wnt/β-catenin signaling pathways and downregulation of levels of autophagy in HUVECs enhanced EndMT was observed, as shown by double immunofluorescence staining assays (**Figure 3A**).

This study further confirmed cell apoptosis levels in Rap, 3MA, BIO, and XAV939 treated groups. Results indicated that Rap and BIO could significantly suppress cell apoptosis. However, 3MA and XAV939 had opposite effects of HUVECs on cell apoptosis, compared with the H_2O_2-induced group. Investigating the molecular mechanisms underlying the function of HUVECs in oxidative stress conditions, this study treated HUVECs for 3 days. This was followed by XAV939 for 1 day before co-treatment with Rap for 2 days. Detection of cell apoptosis

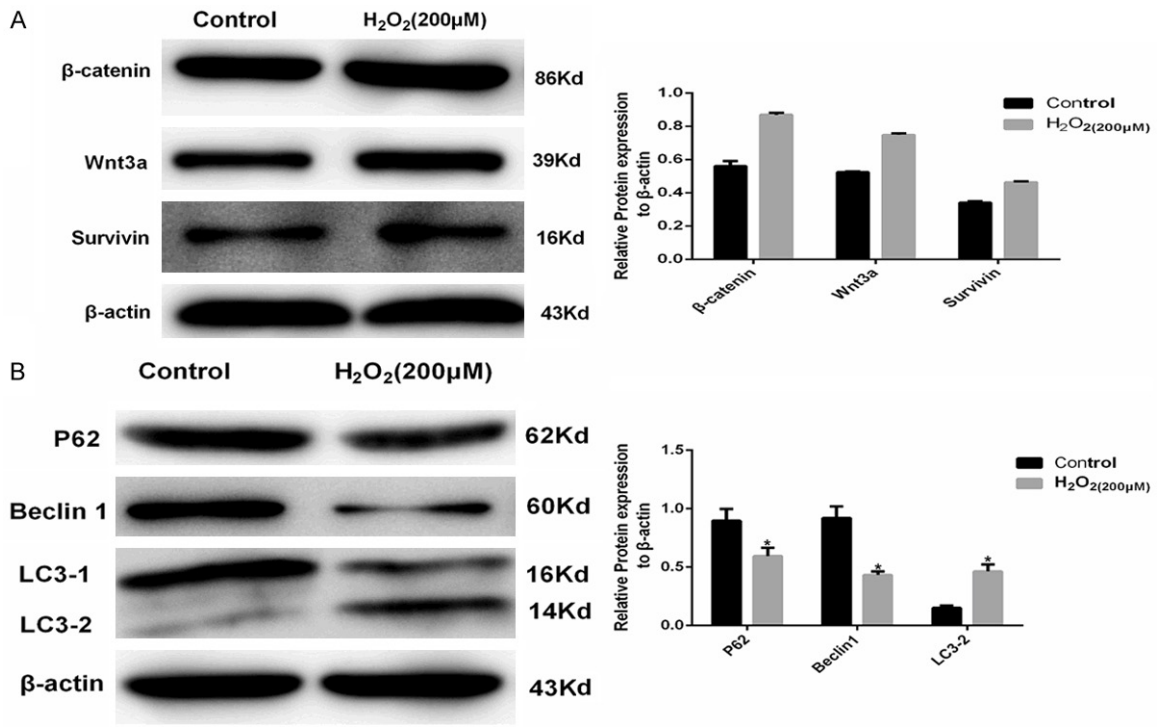
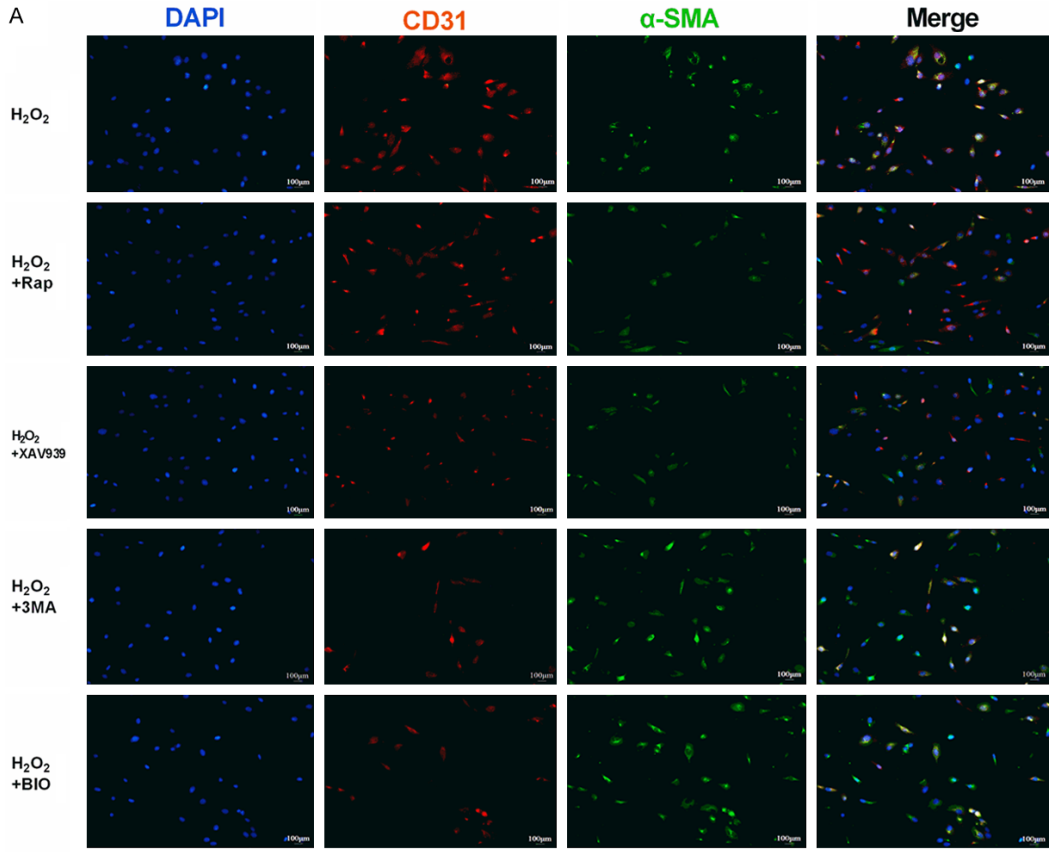


Figure 2. Oxidative stress activates expression of Wnt/β-catenin signaling pathways and promotes autophagy; A. Western blot analysis of Wnt3a, β-catenin, and survivin in HUVECs from each group. β-actin was used as the loading control (*P<0.05 vs. Control group, n=7); B. Western blot analysis of LC3-1/2, Beclin1, and p62 from each group. β-actin was used as the loading control (*P<0.05 vs. Control group, n=7).



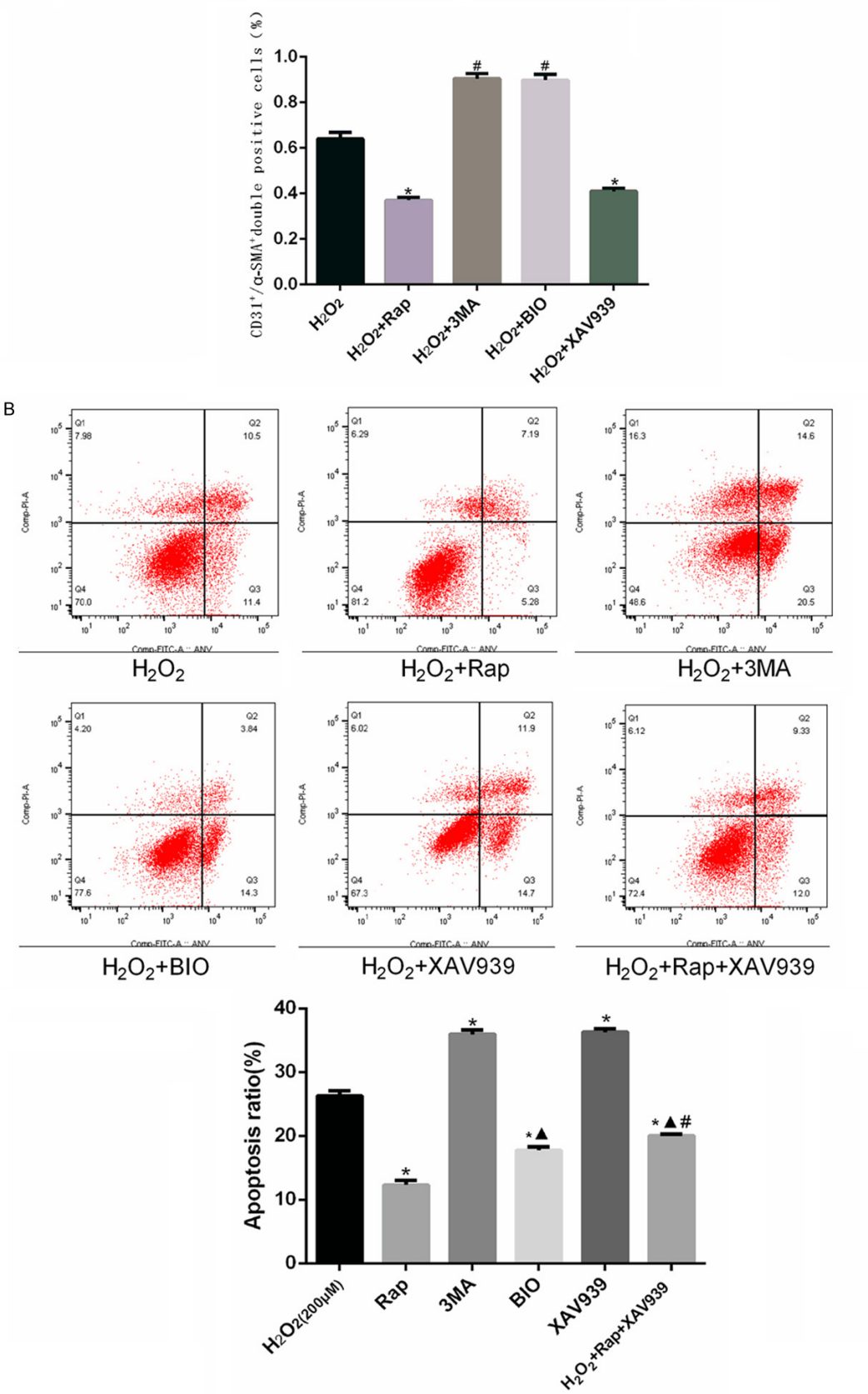

CD31+/α-SMA+double positive cells (%)
1.0
0.8
0.6
0.4
0.2
0.0
H2O2
H2O2+Rap
H2O2+3MA
H2O2+BIO
H2O2+XAV939
B
H2O2
H2O2+Rap
H2O2+3MA
H2O2+BIO
H2O2+XAV939
H2O2+Rap+XAV939
Apoptosis ratio(%)
40
30
20
10
0
H2O2(200μM)
Rap
3MA
BIO
XAV939
H2O2+Rap+XAV939

Figure 3. Modulating levels of autophagic flux and Wnt/β-catenin signaling can partly regulate EndMT and apoptosis in HUVECs under conditions of oxidative stress; A. Double immunofluorescence staining: CD31 (red) and α-SMA (green). Counterstained nuclei with DAPI (blue). Scale bars: 100 μm. Expression of CD31 was lower than controls in the H_2O_2, H_2O_2 + 3MA, H_2O_2 + BIO, and H_2O_2 + Rap + BIO groups. Expression of α-SMA was higher in the H_2O_2, H_2O_2 + 3MA, H_2O_2 + BIO, and H_2O_2 + Rap + BIO groups than the control group. Expression of CD31 was higher and α-SMA was lower in the H_2O_2 + Rap group than the H_2O_2 group (*P<0.05 vs. H_2O_2 group, #P<0.01 vs. H_2O_2 group, n=7); B. Apoptotic cells were measured by annexin V-FITC/PI double staining assay (*P<0.05 vs. H_2O_2, P<0.05 vs. H_2O_2 + Rap, #P<0.05 vs. H_2O_2 + XAV939, n=7).

revealed that the apoptosis rate in group H_2O_2 + Rap + XAV939 was lower than that in the H_2O_2 + XAV939 group, but higher than that in the H_2O_2 + Rap group (**Figure 3B**).

Results suggest that the function of HUVECs under oxidative stress conditions could be modulated by autophagy. Furthermore, the regulatory functions of autophagy might be via Wnt/β-catenin signaling pathways.

Autophagy regulates the function of HUVECs under the conditions of oxidative stress by regulating Wnt/β-catenin signaling pathways

The current study aimed to confirm whether there is cross-talk between autophagy and Wnt/β-catenin signaling pathways, investigating whether the regulatory functions of autophagy for HUVECs in OS are via Wnt/β-catenin signaling. This study treated H_2O_2-induced HUVECs with Rap and Rap + BIO. Autophagy partly weakened the enhancement of EndMT in HUVECS motivated by activating Wnt/β-catenin signaling pathways. The number of $CD31^+$/α-SMA^+ cells in the BIO group was more than that in the Rap + BIO group. Results of immunofluorescence are shown in (**Figure 4A**).

Next, the current study evaluated the interaction of autophagy and Wnt/β-catenin signaling pathways concerning the function of HUVECs under conditions of OS. Western blotting analysis confirmed that relative protein expression levels of Wnt/β-catenin signaling pathways (Wnt3a, β-catenin, survivin) were decreased in the Rap + BIO group, compared to the BIO group. However, levels were increased in the Rap + XAV939 group (**Figure 4B**), compared to the XAV939 group. It was assumed that Wnt/β-catenin signaling pathways were a downstream target of autophagy in modulating the function of HUVECs in OS conditions. At the same time, Western blotting detected Wnt/β-catenin signaling pathway related proteins, indicating that autophagy had inhibitory effects on Wnt/β-catenin signaling pathways.

Discussion

The mechanisms of atherosclerosis have not been thoroughly revealed. Based on previous studies, it was found that oxidative modification of low-density lipoproteins (LDL) into oxidized-LDL (ox-LDL) is an important factor contributing to atherosclerosis in the lipid infiltration theory. Ox-LDL can induce vascular endothelial cells to secrete a variety of inflammatory factors [20]. The chemotaxis aggregation of these inflammatory factors further aggravates the inflammatory reaction of blood vessels. An important part of the inflammation theory is the formation of ox-LDL by oxidative LDL. In addition, hypoxia, oxidative stress, and high-glucose are all stimulant factors leading to endothelial cell injuries [21, 22]. The current study confirmed that the sensitivity and specificity of endothelial dysfunction is the first step toward coronary arteriosclerosis [23]. Results suggest that EndMT not only contributes to atherosclerotic pathobiology but also is associated with complex plaque [24].

OS production, such as ROS, leads to a series of cell damage. Apoptosis is one of these results [25]. During systemic stress, apoptosis participates in the process of self-protection in cells. It is regulated by promoting and inhibiting signals. The initiation phase of apoptosis is when a transmembrane protein, after receiving an apoptosis signal, acts on Fas. It is combined with Fasl to initiate transduction signals of apoptosis [26]. Many inhibitors of apoptosis have been found [27, 28]. In addition to PI3K-AKT-mTOR signaling pathways, Wnt/β-catenin signaling pathways [29, 30] are also involved in the existing mechanisms of apoptosis [31].

HUVECs were put in H_2O_2 to simulate OS conditions. It was observed that apoptosis and EndMT levels were increased in HUVECs. Apoptosis and autophagy are different physiological processes, in which cells maintain their own regeneration and protect themselves from the stress state. Autophagy can prevent endotheli-

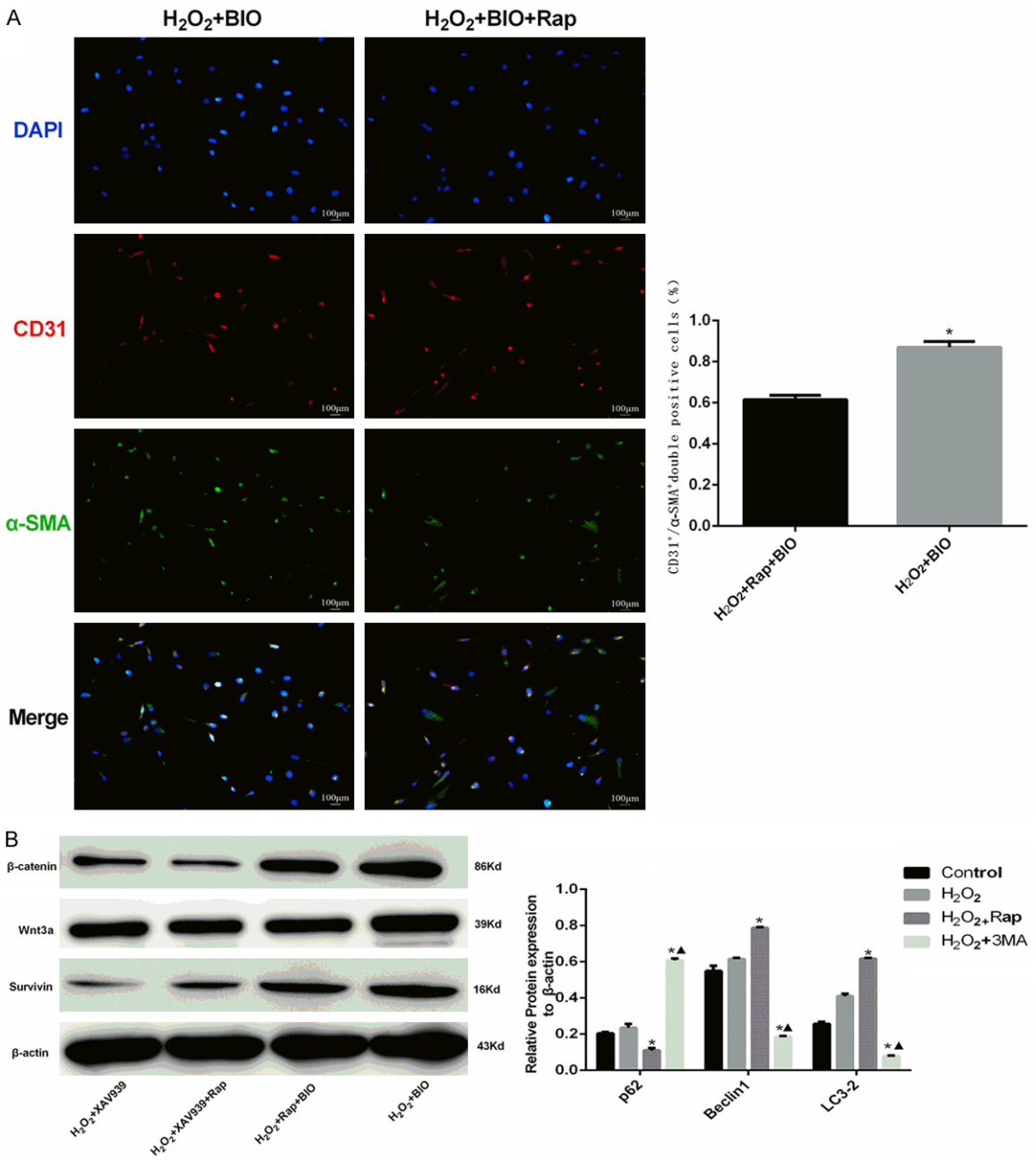


Figure 4. Modulating levels of autophagic flux and Wnt/β-catenin signaling pathways can regulate EndMT and apoptosis in HUVECs. A. Double immunofluorescence staining: CD31 (red) and α-SMA (green). Counterstained nuclei with DAPI (blue). Scale bars: 100 μm. Expression of CD31 was higher and α-SMA lower in the H_2O_2 + Rap + BIO group than the H_2O_2 + BIO group (*P<0.05 vs. H_2O_2 + Rap + BIO group, n=7); B. Western blotting of relative protein expression of Wnt3a, β-catenin, and survivn in the HUVECs from each group (*P<0.05 vs. H_2O_2 + XAV939 group, ▲P<0.05 vs. H_2O_2 + BIO group, n=7).

al cells from entering apoptotic pathways. The current study examined the question of how autophagy regulates the function of HUVECs under conditions of OS and whether expression of Wnt/β-catenin signaling pathway is involved in multiple physiological and pathological processes in cells.

Autophagy is a necessary condition for cells to maintain physiological function [32, 33]. Some abnormal stimulates lead to autophagy inhibition or overdevelopment, which leads to the development of multiple diseases [34]. Autophagy and Wnt/β-catenin signaling pathways respond to this stimulation in the pres-

ence of H_2O_2 mimic OS, with expression levels of both increasing. The current study shows that autophagy is one of the self-protection pathways used in cell metabolism. Thus, levels of autophagy are the initiation of the self-protection mechanisms in cells under the condition of OS. However, the unbalance of autophagy is also a consumption of the cell's own function. Results showed that levels of autophagy and expression of Wnt/β-catenin signaling pathways in HUVECs were increased under OS. Increased expression levels of autophagy and Wnt/β-catenin signaling pathways have a regulatory effect on apoptosis and EndMT for HUVECs under the conditions of OS. This effect is a protective effect on endothelial cells under such conditions.

Vascular endothelial cell structure and function injuries are the motivation of many diseases. Studies have demonstrated that structural and functional damage of endothelial cells is a necessary condition in AS. It leads to chronic ischemia and fibrosis of the viscera and a limp in the limbs. AS is one of the basic pathological changes of coronary heart disease. Thus, the current study explored the regulatory mechanisms and signaling pathways involved in injuries of endothelial cell structure and function under OS.

Present data indicates that improvements of autophagy through Rap can reduce apoptosis rates of HUVECs under OS conditions, as well as inhibit the development of EndMT. Moreover, 3MA inhibition of autophagy increases apoptosis rates of HUVECs under OS and EndMT is more obvious. Activation of Wnt/β-catenin via BIO can reduce apoptosis rates of HUVECs under OS conditions, as well as promote the development of EndMT. However, inhibition of Wnt/β-catenin signaling pathways by XAV939 significantly resulted in an increase of HUVECs apoptosis rates and a decrease of EndMT in OS conditions. Moreover, detection of protein levels showed that autophagy could inhibit expression of Wnt/β-catenin signaling pathways. Based on these results, the interaction between autophagy and Wnt/β-catenin signaling pathways was explored. After BIO and Rap co-treating HUVECs under OS conditions, the promotion effects to the cell development EndMT were reduced, compared with the BIO group, which activated Wnt/β-catenin signaling pathways. After co-treatment with XAV939 and Rap, apoptotic rates of cells were significantly lower than those treated by XAV939 alone.

Results suggest that there is an interaction between autophagy, Wnt/β-catenin signaling pathways, apoptosis, and EndMT in HUVECs under oxidative stress. OS conditions induce HUVECs to develop EndMT. These conditions also promote cell apoptosis, autophagy, and Wnt/β-catenin signal pathways. Levels of autophagy and expression levels of Wnt/β-catenin signaling pathways were regulated. Apoptosis rates and levels of EndMT were changed. Interestingly, expression levels of Wnt/β-catenin signaling pathways were also modulated when autophagy levels were regulated. It is confirmed that autophagy and Wnt/β-catenin signaling pathways have interaction with HUVECs apoptosis and EndMT regulation under OS conditions. Autophagy inhibits Wnt/β-catenin signaling pathways to inhibit HUVECs to develop EndMT under OS conditions. Autophagy inhibits Wnt/β-catenin signal pathways, alleviating apoptosis rates under OS conditions. Autophagy regulates the function of HUVECs under OS via negative regulation of Wnt/β-catenin signaling pathways. The current study may provide a new idea for clinical treatment of cardiovascular diseases, aiming to protect endothelial function under OS conditions and prevent AS.

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Disclosure of conflict of interest

None.

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