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STUDY OF ANGIOTENSINII-ROS-AUTOPHAGY PATHWAY IN VASCULAR ENDOTHELIAL CELLS

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[1]Fan Jun, [1]Yang Chengming, [2]Lian Jiqin, [1]Zeng Chunyu, [2] Ni Zhenhong, [1]Lin Desheng, [1]Ran Xi, [1]Yang Chengming. *[1]Department of Cardiology, Institute of Surgery Research, Daping Hospital, Third Military Medical University, Chongqing 400042, China; [2]Department of Biochemistry and Molecular Biology, Third Military Medical University, Chongqing 400038, China*

Objectives As in recent years cell autophagy in depth research and found that the autophagic degradation of toxic substances, and

damaged proteins and organelles by the lysosome system, and inadequate nutrition, lack of oxygen, recycling of degradation of these substances release of amino acids, nucleotides, fatty acids and other small molecules and energy in order to maintain the steady state of the cell. It is reasoned that the damage caused by Ang II VEC autophagy may be a potential mechanism to prevent the VEC injury, may become a new target for treatment of cardiovascular diseases and new strategies. Current research on the relationship between autophagy of Ang II and the VEC is still rare. In this study, we observed the influence of Ang II on the VEC autophagy, and to investigate the Ang II induced VEC possible mechanisms of autophagy.

Methods Intracellular ROS levels were detected by microplate reader after treatment with Ang II (10^{-7} mol/l) for 24 h in human vascular endothelial cells (EA.hy926). LC3-IIprotein level was detected by western blot assay after stimulation by different concentrations of AngII(10^{-8}, 10^{-7}, 10^{-6} mol/l) or by same concentration (10^{-7} mol/l) for different time (0 h, 6 h,12 h, 24 h, 36 h) in EA cells. The mounts of autophagosomes were evaluated by fluorescence microscope after staining with acridine orange. Similarly, LC3-IIprotein levels and autophagosome formation were detected after treatment with AngII (10^{-7} mol/l), AngII combined 3-MA (2 mmol/l) or AngII combined NAC (50 μmol/l).

Results Treatment with AngII, intracellular ROS levels and LC3-IIprotein level was significantly increased ($p<0.05$) in EA cells, accomplish with the significant increasement of the mounts of autophagosomes. AngII-induced autophagy (both LC3-IIprotein level and autophagosomes) was dramatically down-regulated by 3-MA or NAC in EA cells.

Conclusions AngII induces autophagy through promoting the generation of ROS in EA cells.