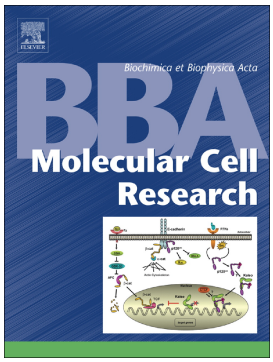

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Low shear stress induces vascular eNOS uncoupling via autophagy-mediated eNOS phosphorylation

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Abstract Uncoupled endothelial nitric oxide synthase (eNOS) produces $O_2^{\cdot-}$ instead of nitric oxide (NO). Earlier, we reported rapamycin, an autophagy inducer and inhibitor of cellular proliferation, attenuated low shear stress (SS) induced $O_2^{\cdot-}$production. Nevertheless, it is unclear whether autophagy palys a critical role in the regulation of eNOS uncoupling. Therefore, this study aimed to investigate the modulation of autophagy on eNOS uncoupling induced by low SS exposure. We found that low SS induced endothelial $O_2^{\cdot-}$burst, which was accompanied by reduced NO release. Furthermore, inhibition of eNOS by L-NAME conspicuously attenuated low SS-induced $O_2^{\cdot-}$releasing, indicating eNOS uncoupling. Autophagy markers such as LC3 II/I ratio, amount of Beclin1, as well as ULK1/Atg1 were increased during low SS exposure, whereas autophagic degradation of p62/SQSTM1 was markedly reduced, implying impaired autophagic flux. Interestingly, low SS-induced NO reduction could be reversed by rapamycin，WYE-354 or ATG5 overexpression vector via restoration of autophagic flux, but not by N-acetylcysteine or apocynin. eNOS uncoupling might be ascribed to autophagic flux blockade because phosphorylation of eNOS Thr495 by low SS or PMA stimulation was also regulated by autophagy. In contrast, eNOS acetylation was not found to be regulated by low SS and autophagy. Notably, although low SS had no influence on eNOS Ser1177 phosphorylation, whereas boosted eNOS Ser1177 phosphorylation by rapamycinwere in favor of the eNOS recoupling through restoration of autophagic flux. Taken together, we reported a novel mechanism for regulation of eNOS uncoupling by low SS via autophagy-mediated eNOS phosphorylation, which is implicated in geometrical nature of atherogenesis.

Keywords—Autophagic flux, Low shear stress, Endothelial cells, Endothelial nitric oxide synthase uncoupling

Introduction

Endothelial nitric oxide synthase (eNOS), located in membrane caveolae or on Golgi apparatus, serves as a critical enzyme in maintaining vascular hemostasis. However, under pathological conditions such as atherosclerosis, hypertension, diabetes, ischemia/reperfusion injury and smoking, eNOS becomes unstable and uncoupled as presented by production of $O_2^{\cdot-}$rather than nitric oxide (NO). [1]Molecular mechanisms of eNOS uncoupling include deficiency of tetrahydrobiopterin (BH4) or BH4 oxidation, reduced intake of L-arginine, as well as posttranslational modification of eNOS, especially phosphorylation of eNOS Thr495 residue. [2] The latter is deemed as a 'switch' of eNOS uncoupling. [3] Shear stress (SS) is the friction force imposed by blood flow on endothelial surface. Physiological SS, ranged of 10-70dyne/cm^2 in arterial system, is atheroprotective, while low SS (less than 10 dyne/cm^2) is implicated in atherosclerotic lesions embedment on arterial bifurcation or tortuous arteries.[4] eNOS-derived