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The Role of Autophagy in the Differentiation of EPCs Induced by Shear Stress

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Abstract: Aims: Endothelial progenitor cells (EPCs) play an important role in postnatal angiogenesis and neovascularization. Previous studies have revealed shear stress could accelerate EPC proliferation, differentiation, migration and so on, which contribute to postnatal angiogenesis and neovascularization. Moreover, some studies indicate that autophagy actively participates angiogenesis by affecting EPC migration and differentiation. Here, we try to elucidate the possible roles of autophagy of EPC differentiation induced by shear stress. **Methods and Results:** EPCs were exposed to shear stress (12 dyne/cm^2). And then the expression of autophagy markers, such as LC3II/I, P62andATG5, were analyzed using Western blot. The results have shown that in EPCs, shear stress triggered an increase in LC3II/I and ATG5 at 10 min, which was then followed by a decrease. In contrast, shear stress caused a decrease in P62 at 10 min, which was then followed by an increase. Furthermore, immunostaining revealed that the unsheared cells showed only weak LCII staining. However, shear stress increased LCII staining. Bafromycin experiment confirmed that the increase of autophagy caused by shear stress was due to an increase in the formation of autophagy rather than a decrease in the degradation of autophagosomes. To examine the role of autophagy in the shear stress-induced EPC differentiation, we pretreated late EPCs with 3-MA, an inhibitor of autophagy, before the application of shear stress. Through real time RT-PCR and FACS analyses, we observed that the pretreatment of EPCs with 3-MA significantly inhibited the shear stress induced up-regulation of vWF and CD31. In the mean time, treatment of EPCs with LY294002 (a small molecule inhibitor of PI3K) or KLF2 siRNA inhibited the shear stress-induced EPC autophagy and differentiation. **Conclusion:** Autophagy is involved in the shear stress-upregulated expression of endothelial markers vWF and CD31 in EPCs. Moreover, this increase was observed to be mediated by PI3K and KLF2. Although further studies are needed to confirm the relationship between these mechanosensitive molecules, the present results may provide new insights into the relationship between EPC autophagy and differentiation induced by shear stress.

Keywords: Endothelial progenitor cell, shear stress, autophagy, differentiation.

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