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Autophagy in the endothelium commands flow-mediated vascular reactivity and remodelling, and regulates VEGFR2 subcellular localization and signalling

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ABSTRACT

Pharmacological approaches aimed at increasing autophagic flux and genetically engineered mice with autophagy deficiency in the endothelium have demonstrated that autophagy exerts vessel protection against metabolic stresses and vascular aging. However, the identity of the specific cellular processes that autophagy controls in endothelial cells remained unclear. In this punctum, we discuss our recent findings on the multiple functions of autophagy in the endothelium. Particularly, we highlighted that autophagy controls flow-mediated vascular reactivity and remodeling. We have also focused on the role of autophagy machinery in regulating protein distribution within the cell and on the results demonstrating how autophagy modulates the cellular response to the microenvironment changes.

Abbreviations: CXCL12: C-X-C motif chemokine ligand 12 EDN1: Endothelin 1 eNOS: endothelial Nitric Oxide Synthase FGF: Fibroblast Growth Factor NO: Nitric oxide VEGF: Vascular Endothelial Growth Factor VEGFR2: Vascular Endothelial Growth Factor Receptor 2

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Our recent findings support the notion that autophagy not only degrades intracellular components but also effectively participates in maintaining endothelial cell homeostasis, and impairment of endothelial cell autophagy is the basis for endothelial dysfunction with consequences for the entire body. Autophagy stabilizes mitochondrial functions, protects against oxidative stress, regulates cell metabolism, maintains cell signaling, regulates cell-cell junctions, enhances angiogenesis, and coordinates endothelial cell adaptation to the microenvironment [1].

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Ten years ago, pioneering experiments linked the regulation of autophagy to vascular function by showing that inhibition of autophagy in endothelial cells *in vitro* decreased VEGF-mediated angiogenesis. Since then, several studies have confirmed that autophagy can regulate vascular functions, associating autophagy impairments with vascular diseases. However, the molecular mechanisms regulated by autophagy in endothelial cells that allow to have this vasculoprotective effects have not been described yet. Therefore, our study aimed to fill this knowledge gap. We used transcriptomic and proteomic methods to determine the effects of autophagy deficiency in endothelial cells. These methods revealed that autophagy deficiency leads to mitochondrial dysfunction, changes in cell metabolism, senescence, and activation of the cellular response to stress such as the EIF2 pathway. The mitochondrial dysfunction was further confirmed by immunofluorescence, Western blot and assessment of the mitochondrial healthy pool by flow cytometry. Measurement of metabolites also highlighted changed in the tricarboxylic acid cycle. Considering the established role of autophagy in regulating mitochondrial pool, endoplasmic reticulum (ER) recycling, and response to environmental stress, it is not entirely unexpected that the lack of autophagy in endothelial cells would result in such alterations. Then, we proceeded to investigate the contents of autophagosomes, with the aim of shedding light on other potential functions of autophagy.

Indeed, classically, proteins are regulated at three levels: transcription, translation, and post-translation. However, a fourth and a fifth level also exist: subcellular localization and degradation. Given that autophagy is a proteostatic pathway, we aimed at determining whether it could regulate endothelial cell function by recycling specific proteins. To address this question, we analyzed the autophagosome content using proteomics. This approach enabled us to identify other functions of endothelial autophagy that transcriptomic and proteomic approaches have not yet identified. It was expected that the proteins degraded by autophagy would be in higher amounts in cells in which this pathway was inhibited. Surprisingly, the total cellular levels of most of the proteins contained in the autophagosomes were not reduced. Therefore, we hypothesized that the autophagy machinery, beyond protein recycling, plays a role in intracellular protein trafficking and localization. We highlighted that the autophagy machinery plays a key role in the localization of some proteins in cells, which is crucial for proper cell signal transduction. Notably, we showed by immunofluorescence that *autophagy-related 5 (ATG5)* deficiency in endothelial cells impairs VEGFR2 localization to the cell surface, and consequently, Western blot analysis demonstrated that VEGFR2 downstream signaling is reduced leading to a decreased eNOS activation. We showed that the consequence of such dysregulation is physiologically

important since endothelial-selective *atg5*^{-/-} mice show lower plasmatic NO level, impaired flow-induced vasodilation measured *in vivo* in the basilar trunk artery and *ex vivo* in isolated kidneys and mesenteric arteries, exacerbated hypertension in response to vasoconstrictive molecules, inadequate response to flow-mediated remodeling, post-injury neointima formation and hemodynamic maladaptation to post-myocardial infarction. Taking advantage of such a gene-targeting strategy, we identified the role of endothelial autophagy in regulating flow-mediated dilation and vascular remodeling *ex vivo* and *in vivo*.

Another major finding of our study is the decreased angiogenic capacity *in vitro* and *in vivo* in *ATG5*-deficient endothelial cells. This involved impaired VEGF-VEGFR2 signaling, but impaired neo-angiogenesis in response to FGF was also observed, suggesting that autophagy could regulate other growth factor signaling cascades as well.

However, our study focused on VEGF-VEGFR2 signaling. We detected 63 cell surface proteins in autophagosomes from endothelial cells by proteomics, including several tyrosine kinase receptors and 36 extracellular proteins. Most of these extracellular and membrane proteins belong to families of proteins with key regulatory functions in endothelial cells such as proteins of the CXCL12-, EDN1, TGF β , Angiopoietin-, Netrin-, VEGF-, Notch-, or Semaphorin protein families. Thus, the autophagy machinery may control endothelial cell functions by regulating the cellular trafficking of other cell surfaces and secreted proteins beyond VEGFR2. Moreover, in addition to its canonical role in recycling organelles and proteins, the autophagy machinery could be an essential routing platform for extracellular and membrane proteins, thereby regulating endothelial sensing of extracellular signals. Supporting this notion, a previous study demonstrated that endothelial *ATG7* deficiency in mice induced an increased bleeding time due to a defect in the maturation of the von Willebrand factor, linking the autophagy machinery to Weibel-Palade bodies, which are storage granules in endothelial cells. Likewise, we confirmed that endothelial *ATG5* deficiency also causes hemostasis defects in mice, and detected several proteins of Weibel-Palade bodies within autophagosomes.

In conclusion, our results demonstrate the pivotal role of autophagy in the endothelial function of small vessels, impacting fundamental physiological processes. Significantly, flow-dependent vasodilation is frequently blunted in asymptomatic individuals with coronary artery diseases, even when acetylcholine-mediated dilation is normal. Our findings suggest that a chronic autophagy impairment in the endothelium may contribute to endothelial dysfunction and cardiovascular diseases.

Conflict of interest

The authors have nothing to declare.

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