Autophagy

Autophagy

ISSN: 1554-8627 (Print) 1554-8635 (Online) Journal homepage: http://www.tandfonline.com/loi/kaup20

Cellular processes underlying cerebral cavernous malformations: Autophagy as another point of view

Saverio Marchi, Saverio Francesco Retta & Paolo Pinton

To cite this article: Saverio Marchi, Saverio Francesco Retta & Paolo Pinton (2016) Cellular processes underlying cerebral cavernous malformations: Autophagy as another point of view, Autophagy, 12:2, 424-425

To link to this article: http://dx.doi.org/10.1080/15548627.2015.1125073

Published online: 22 Feb 2016.

AUTOPHAGY
2016, VOL. 12, NO. 2, 424–425
http://dx.doi.org/10.1080/15548627.2015.1125073

Taylor & Francis
Taylor & Francis Group

AUTOPHAGIC PUNCTUM

Cellular processes underlying cerebral cavernous malformations: Autophagy as another point of view

Saverio Marchi[a], Saverio Francesco Retta[b], and Paolo Pinton[a]

[a]Department of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology, University of Ferrara, Ferrara, Italy; [b]Department of Clinical and Biological Sciences, University of Torino, Torino, Italy

ABSTRACT
A growing amount of evidence indicates that autophagy plays a pivotal role in a plethora of human pathological conditions. We have recently broadened the list of the so-called autophagy-related diseases, describing the involvement of defective autophagy in the pathogenesis of cerebral cavernous malformations.

ARTICLE HISTORY
Received 9 November 2015
Revised 18 November 2015
Accepted 20 November 2015

KEYWORDS
autophagy; CCM; endothelial-to-mesenchymal transition (EndMt); MTOR; pharmacological therapy; ROS

Cerebral cavernous malformations (CCMs; OMIM: 116860) consist of conglomerations of sinusoidal capillaries in the brain with enlarged and irregular architecture. These abnormal vascular structures are characterized by a thin endothelium devoid of normal vessel wall components, and may result in intracerebral hemorrhage and consequent pathological conditions. CCMs may arise sporadically or can be inherited as an autosomal dominant condition with incomplete penetrance and variable expressivity. Loss-of-function mutations of 3 different genes are associated with CCMs: *KRIT1/CCM1* (KRIT1, ankyrin repeat containing), *CCM2* (*MGC4607*) and *PDCD10/CCM3* (programmed cell death 10). Approximately 30% of people with CCMs experience clinical symptoms, such as seizures, strokes, headaches, and focal neurological deficits. To date, treatment of CCM lesions is mainly limited to neurosurgery; however, this is not always practicable, especially for multiple CCMs or CCMs located in sensitive areas of the brain. Therefore, novel pharmacological approaches are required for preventing and treating this disease, which should derive from a comprehensive understanding of molecular mechanisms underlying CCM pathogenesis.

Over the past few years, several molecular pathways have been associated with the genesis and progression of CCM lesions, including but not limited to the RHO-ROCK, TGFB/TGF-β/BMP, CTNNB1/β-catenin and NOTCH pathways, as well as distinct redox molecular routes modulated by reactive oxygen species (ROS). In our study, we showed that downregulation of the *KRIT1* gene induces defects in autophagic degradation, evidenced by increased levels of both SQSTM1/p62 and MAP1LC3B proteins. Furthermore, we observed accumulation of aggresome-like structures, as well as increased amounts of SQSTM1-positive bodies in detergent-insoluble fraction, reflecting the inefficiency of protein aggregate clearance by the autophagic machinery. These findings have been obtained both in multiple KRIT1-silenced endothelial cell lines and KRIT1-knockout (KO) fibroblasts, suggesting that insufficient autophagy occurred in a cell-autonomous manner.

But what about the molecular mechanisms underpinning KRIT1-dependent autophagy dysregulation? When analyzing the appearance of KRIT1 KO cells, we observed an increase in size and cell proliferation rate, 2 typical traits that can be connected to MTOR (mechanistic target of rapamycin [serine/threonine kinase]) activity. Indeed, KRIT1-depleted cells display hyperactivation of the MTOR pathway, with consequent reduced activity of ULK1, one of the major targets of MTOR-dependent autophagy regulation. Both Torin1 and rapamycin, 2 major MTOR inhibitors, reestablish, at least in part, autophagy in KO cells, whereas treatment with xestospongin B, an MTOR-independent pro-autophagic stimulus, fails to restore the correct autophagic flux. Altogether, these results demonstrate that the defective autophagy observed upon KRIT1 loss is mainly due to MTOR activity.

The 3 proteins implicated in this disease may interact to form the "CCM complex," which is involved in the maintenance of vascular homeostasis. Moreover, mutations in any of the 3 *CCM* genes lead to the onset of similar pathological signatures, suggesting that the 3 CCM proteins share a common mechanism of action or that the physiological activity of the complex is strictly dependent on the correct function of its components. Thus, we investigated if downregulation of CCM2 and PDCD10 expression would have the same impact on the autophagic process. Both CCM2-silenced and PDCD10-deficient endothelial cells exhibit SQSTM1 and MAP1LC3B accumulation due to MTOR upregulation. Importantly, analysis of retinas derived from an endothelial-specific *Pdcd10* knockout mouse model, which presents malformations at the periphery of retinal vascular plexus, display SQSTM1 clusters in endothelial cells forming the vascular lesions. Similar results have been obtained in surgical samples of human

CONTACT Saverio Francesco Retta francesco.retta@unito.it; Paolo Pinton paolo.pinton@unife.it
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/kaup.

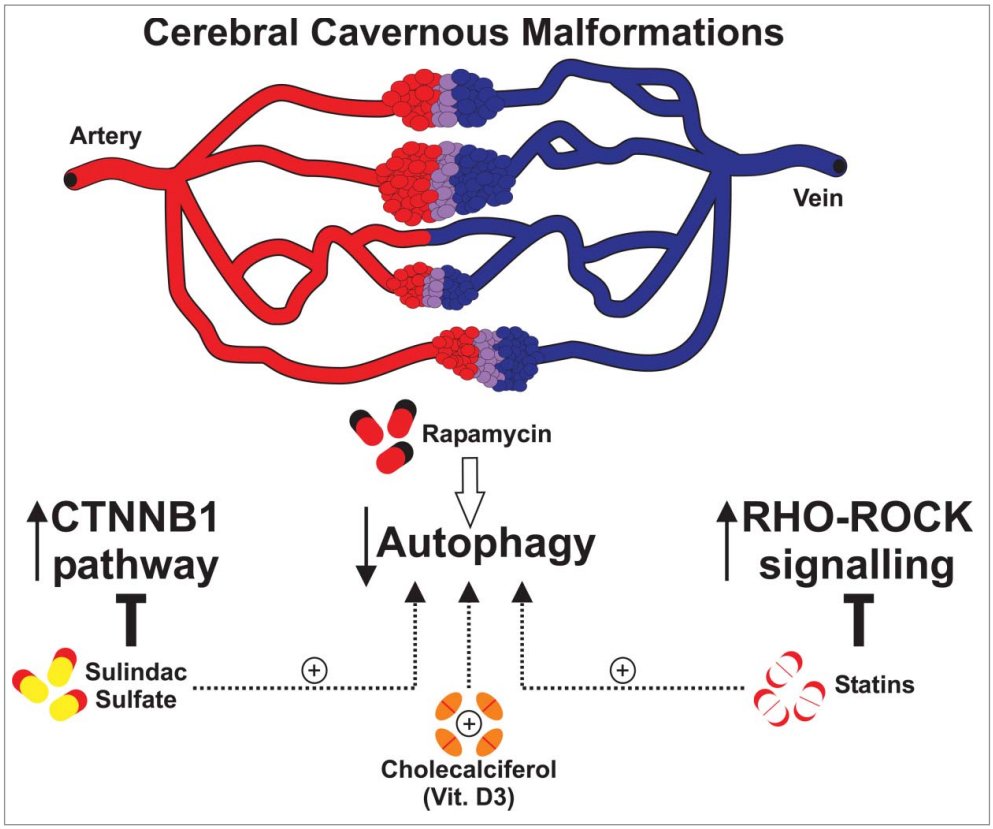

Figure 1. Role of autophagy in cerebral cavernous malformations. CCMs are agglomerations of capillaries in the brain consisting of clustered, enlarged structures. The figure illustrates some of the different molecular pathways that are altered upon loss-of-function mutation of CCM-associated genes. These include increased CTNNB1/β-catenin and RHO-ROCK signaling, as well as suppression of the autophagic process through upregulation of MTOR. Therapeutic approaches depicted here, have been also reported to trigger autophagy by MTOR inhibition. Please refer to the text for further explanation.

CCM lesions. Further studies are needed to identify the molecular mechanism governing MTOR activation upon malfunctioning of the CCM complex.

Is insufficient autophagy only a phenomenon that occurs during CCMs or could it regulate other pathological aspects that contribute to CCM progression? To address this relevant question, we explored the correlation between autophagy and 2 major pathological signatures of the disease, such as endothelial-to-mesenchymal transition (EndMt) and ROS production. Treatment with MTOR inhibitors reduces expression of mesenchymal markers and augments the amounts of endothelial markers, as well as restores cell migration to physiological levels. In addition, pharmacological inhibition of MTOR also decreases mitochondrial ROS production in KRIT1-KO cells. Importantly, administration of antioxidants, such as N-acetyl cysteine, reduces intracellular ROS levels but is unable to reactivate autophagy or minimize the upregulated MTOR signaling pathway occurring upon KRIT1 depletion. Moreover, *ATG7* silencing in endothelial cells induces a mesenchymal switch, evidenced by slow formation of capillary-like structures, increased migratory capacity, and gain of specific biomarkers. Thus, the MTOR-dependent inhibition of autophagy is closely related to both EndMt and ROS accumulation, suggesting a causative link.

Could defective autophagy be considered one of the main routes leading to irregular endothelial growth and thus a promising target for a therapeutic approach? Recent findings demonstrate that endothelial cell-specific knockdown of the *ATG5* gene induces an increase in structural abnormalities of tumor vasculature that are similar to those characterizing CCM vessels, including the formation of large and leaky sinusoids. Most importantly, different compounds proposed as noninvasive drug treatment approaches for CCMs, including statins (e.g., Simvastatin), cholechalciferol (vitamin D3), and nonsteroidal anti-inflammatory drugs (Sulindac sulfide and its analogs) have been also described as pro-autophagic stimuli that function by inhibiting the MTOR pathway (Fig. 1). Conversely, suppression of autophagy might constitute a side effect that limits the efficacy of some pharmacological therapies. This might be the case for the nonselective β-adrenergic antagonist Propranolol, which has been suggested as a potential candidate for treating CCMs, but also as an inhibitor of autophagic flux and mild activator of MTOR, especially at high dosage. Overall, employment of MTOR inhibitors, such as rapamycin, alone or in combination with adjuvant therapies, could represent a novel valuable strategy for the treatment of CCM disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by a grant from Telethon (GGP15219).