Editorial

The AMPK-SKP2-CARM1 axis links nutrient sensing to transcriptional and epigenetic regulation of autophagy

Ziheng Xu, Daniel J. Klionsky

Life Sciences Institute and Department of Molecular, Cellular and Developmental Biology, University of Michigan Ann Arbor, MI, USA
Correspondence to: Daniel J. Klionsky. University of Michigan, Life Sciences Institute, Rm. 6036, 210 Washtenaw Ave., Ann Arbor, Michigan 48109-2216, USA. Email: klionsky@umich.edu.

Submitted Aug 12, 2016. Accepted for publication Aug 15, 2016.
doi: 10.21037/atm.2016.08.68
View this article at: http://dx.doi.org/10.21037/atm.2016.08.68

Macroautophagy/autophagy is a highly conserved catabolic process that targets bulk cytoplasm, and damaged organelles or other harmful intracellular content to the lysosome/vacuole for degradation. Autophagy is essential for maintaining proper cellular homeostasis and for survival under adverse conditions. When this cytoprotective process becomes dysfunctional, it is often associated with a spectrum of human ailments including cancer and neurodegenerative diseases. Intensive studies have been carried out in the past two decades to understand the mechanism and regulation of autophagy; so far, more than thirty autophagy-related (*ATG*) genes have been identified in human that orchestrate the complex membrane dynamics involved in autophagic sequestration. In order to further our understanding of this crucial cellular activity, and to gain the knowledge necessary to modulate autophagy for therapeutic purposes, it is imperative that we continue to explore the mechanisms involved in its regulation.

Autophagy involves the sequestration of cytoplasm via a double-membrane intermediate structure termed the phagophore, which matures into an autophagosome; the latter compartment fuses with a lysosome allowing degradation and recycling of the cargo (1). The process of phagophore expansion provides tremendous flexibility and capacity with regard to cargo, allowing entire organelles to be eliminated via autophagy; however, this flexibility also means that autophagy must be tightly regulated in order to prevent inappropriate degradation, which could lead to cell death (2). Given this potential for harming the cell, and the importance of autophagy in homeostasis and response to stress, the cell utilizes a range of mechanisms to regulate this process at different steps and to ensure that it is finely tuned. In addition to the cytoplasmic post-translational modification of various ATG proteins, recent studies have delved into the transcriptional and epigenetic control of autophagy (3). Notably in human cells, TFEB (transcription factor EB) and ZKSCAN3 (zinc finger with KRAB and SCAN domains 3) have been implicated in playing a central role in autophagy regulation (4,5). Accumulating evidence has also suggested histone modification/DNA methylation as an alternative approach for long-term autophagy control (6). Recently, Shin and colleagues reported a new AMPK-SKP2-CARM1 [AMP-activated protein kinase; S-phase kinase-associated protein 2 (p45); coactivator-associated arginine methyltransferase 1] regulatory axis that incorporated cellular nutrient sensing with transcriptional as well as epigenetic control of autophagy (7).

Shin *et al.* started off by noticing an increase in histone H3 arginine 17 dimethylation (H3R17me2) in response to autophagy induced by either nutrient starvation or treatment with rapamycin, an inhibitor of the primary negative regulator of autophagy, MTOR, in mouse embryonic fibroblasts (MEFs). Interestingly, the protein level of the methyltransferase responsible for this histone modification, CARM1, is also upregulated upon nutrient starvation. The authors utilized knockout and enzymatic activity-deficient knockin mutants of CARM1 and observed in both cell lines a deficit in autophagy induction as well as autophagic degradation, monitored by lipidation of LC3 (a marker protein of autophagosomes that is covalently attached to phosphatidylethanolamine) and degradation of the autophagic receptor SQSTM1/p62, respectively. LC3 flux (that is, its ultimate degradation within the lysosome resulting from its role in binding cargo receptors), autophagosome formation and maturation are compromised as well in *Carm1* knockout and activity-deficient cell lines.

Now that CARM1 was observed to have an established role in autophagy, the authors went on to determine how this protein could be potentially regulated. The induction of CARM1 is confined within the nucleus and is repressed after treatment with MG132, a 26S proteasome inhibitor. These findings indicate that proteasomal degradation can be a major regulatory pathway of CARM1. After glucose starvation, the authors reported a decreased ubiquitination of CARM1, which is achieved by the downregulation of the specific SKP2-containing SCF E3 ubiquitin ligase complex. This relationship was further corroborated by the fact that SKP2 depletion decreases CARM1 ubiquitination and thus extends its half-life. In contrast, overexpression of SKP2 but not the SKP2 mutant that is deficient in complex formation, results in increased CARM1 ubiquitination.

Thus far, the data from Shin *et al*. demonstrated that glucose starvation is responsible for reducing the SKP2-containing SCF E3 ligase expression level and therefore diminishing the proteasomal degradation of CARM1, a methyltransferase that contributes to autophagy induction. The next problem, however, was determining the factors that controlled the decrease of SKP2. The authors decided to focus downstream from the initial nutrient sensing pathways, among which one of the most significant involves AMPK (8). A *prkaa1*$^{-/-}$*/ampkα1 prkaa2*$^{-/-}$*/ampkα2* double knockout strain shows less reduction in SKP2 upon glucose starvation, and the introduction of wild-type *PRKAA2* rescues the phenotype. However, no evidence was found regarding a direct interaction between AMPK and SKP2. In addition, the reduction of SKP2 during starvation was shown to be transcriptional instead of post-translational. Due to the presence of a FOXO response element in the *Skp2* promoter, the authors postulated that FOXO3, a transcription factor that is a downstream target of AMPK, might function as the key mediator connecting this nutrient sensor and transcriptional inhibition of SKP2. Accordingly, they performed a luciferase reporter assay using cells in which luciferase expression is driven by the wild-type *Skp2* promoter, or the same promoter with a mutation in the FOXO response element. They found decreased transcription under conditions of glucose starvation in the former, but not the latter. Along these lines, a *foxo1*$^{-/-}$ *foxo3*$^{-/-}$ *foxo4*$^{-/-}$ triple knockout strain is defective in lowering the *Skp2* mRNA level.

Trying to further examine the role that the epigenetic regulator CARM1 plays in autophagy, Shin and colleagues performed RNA-seq and ChIP-seq analyses on wild type and CARM1-depleted cells. They found an increase in H3R17me2 and H3K4me3 levels on the promoters of a set of target genes, which further belong to the set of autophagy and lysosomal genes regulated by TFEB. Given the intranuclear mutual binding between CARM1 and TFEB, the authors proposed that CARM1 functions as a co-activator of TFEB. Knocking out *Carm1* results in a decrease in transcription of a subset of genes regulated by TFEB, and a lower H3R17me2 level of target gene promoters. TFEB overexpression in a *Carm1* knockout mutant fails to rescue the resulting autophagy deficit. Moreover, a TFEB-depleted strain shows an inhibition of CARM1 recruitment to the target gene promoters, and reduced H3R17 methylation. The authors substantiated the role of CARM1 as a co-activator of TFEB by observing respective changes in *ATG* gene transcription when CARM1 activity is compromised.

Ellagic acid, a naturally occurring polyphenol antioxidant, is capable of inhibiting the H3R17me2 modification, and thus autophagy, both *in vitro* and *in vivo*. After validating the presence of the CARM1-dependent regulation axis in mouse hepatocytes, Shin and colleagues additionally tested the inhibitory effect of ellagic acid *in vivo*. Treatment with ellagic acid significantly blocks the recruitment of CARM1 to its target genes, and leads to reduced expression of CARM1-dependent *ATG* and lysosomal genes.

In conclusion, this AMPK-SKP2-CARM1 signaling axis integrates the various levels of autophagy regulation including cell signaling, and transcriptional regulation as well as epigenetic modification. Epigenetic and transcriptional regulation provides an energy-saving approach for control and also create an enduring memory in preparation for future adverse events. Thus, this study has deepened our understanding of how autophagy can be controlled in a holistic manner by pathways linking a multitude of regulation mechanisms. Given the extensive involvement of autophagy in human diseases, this work also presents potential directions for novel therapeutic intervention.

Acknowledgements

Funding: This work was supported by the National Institute of Health (GM053396 to D.J.K.).

Footnote

Provenance: This is a Guest Editorial commissioned by Section Editor Hongzheng Meng, MD, PhD (Department

of Orthopedics, the First Affiliated Hospital of China Medical University, Shenyang, China).
Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Shin HJ, Kim H, Oh S, *et al*. AMPK-SKP2-CARM1 signalling cascade in transcriptional regulation of autophagy. Nature 2016;534:553-7.

References

1. Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. Nat Cell Biol 2010;12:814-22.
2. Mizushima N, Levine B, Cuervo AM, et al. Autophagy fights disease through cellular self-digestion. Nature 2008;451:1069-75.
3. Füllgrabe J, Klionsky DJ, Joseph B. The return of the nucleus: transcriptional and epigenetic control of autophagy. Nat Rev Mol Cell Biol 2014;15:65-74.
4. Settembre C, Di Malta C, Polito VA, et al. TFEB links autophagy to lysosomal biogenesis. Science 2011;332:1429-33.
5. Chauhan S, Goodwin JG, Chauhan S, et al. ZKSCAN3 is a master transcriptional repressor of autophagy. Mol Cell 2013;50:16-28.
6. Taipale M, Rea S, Richter K, et al. hMOF histone acetyltransferase is required for histone H4 lysine 16 acetylation in mammalian cells. Mol Cell Biol 2005;25:6798-810.
7. Shin HJ, Kim H, Oh S, et al. AMPK-SKP2-CARM1 signalling cascade in transcriptional regulation of autophagy. Nature 2016;534:553-7.
8. Kim J, Kundu M, Viollet B, et al. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011;13:132-41.

Cite this article as: Xu Z, Klionsky DJ. The AMPK-SKP2-CARM1 axis links nutrient sensing to transcriptional and epigenetic regulation of autophagy. Ann Transl Med 2016;4(Suppl 1):S7. doi: 10.21037/atm.2016.08.68

