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Autophagy regulation depends on ER homeostasis controlled by lipid droplets

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ABSTRACT

Macroautophagy (hereafter autophagy) is a highly conserved homeostasis and quality control process critically linked to neurodegeneration, metabolic diseases, cancer, and aging. A key feature of autophagy is the de novo formation of autophagosomes, double-membrane vesicular structures encapsulating cytoplasmic cargo for vacuolar turnover and recycling. The membrane rearrangements underlying nucleation, expansion, closure, and vacuolar fusion of autophagosomes are driven by multicomponent core autophagy machinery in cooperation with numerous factors involved in a variety of cellular processes. Our current understanding of the origin and contribution of diverse membrane sources to autophagosome biogenesis and of cellular functions enabling stress-appropriate autophagy responses critical for cell health and survival remains limited. Here, we summarize and discuss our recent findings analyzing the role of lipid droplets (LDs), conserved intracellular storage compartments for neutral lipids, for autophagy regulation. Our data indicate that LDs are dispensable as membrane sources, but fulfill critical functions for maintaining endoplasmic reticulum (ER) homeostasis, including buffering of newly synthesized fatty acids and maintenance of phospholipid composition, required for intact autophagy regulation and cell survival during nutrient stress.

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In our recently published work, we dissected the role of LDs as potential membrane sources for the formation of autophagosomes and in maintaining ER homeostasis for autophagy regulation. Toward this goal, we used *Saccharomyces cerevisiae* as a model system and generated a strain carrying deletions in the 4 genes required for neutral lipid synthesis, which therefore is devoid of LDs, and assessed its ability to perform autophagy. We found that the absence of LDs conditionally affects autophagy and ER homeostasis. Upon nitrogen starvation (starvation), LD-deficient cells show severely compromised autophagy flux due to defective autophagosome biogenesis, characterized by a drastically reduced number of detectable autophagosomes and an unusual formation of multiple Atg8-marked puncta per cell. As a physiological consequence, cells lacking LDs display a substantially shortened life span during starvation. In contrast, pharmacological inhibition of TORC1 by rapamycin elicits a wild-type-like autophagy response in LD-deficient cells. Together, these observations challenged the view on LDs as obligatory membrane sources and pointed to a regulatory role of LDs for starvation-induced autophagy.

Given the close functional relationship of LDs with the ER in lipid metabolism and the central role of the ER for autophagy, we probed for effects of LD-deficiency on ER homeostasis. First, we found that LD-deficient cells constitutively activate unfolded protein response signaling consistent with chronic ER stress. Second, the interconnected tubular and sheet-like morphology of the ER collapses into a simplified network of continuous and dilated tubules in the absence of LDs during starvation. Our work linked these changes in ER morphology and defective autophagosome formation to the absence of LD-mediated buffering of de novo fatty acid synthesis. Specifically, selectively inhibiting fatty acid synthesis or improving fatty acid resistance of LD-deficient cells by genetically uncoupling phospholipid biosynthesis and expanding the ER prevents chronic ER stress and alterations in ER morphology, and partially restores autophagosome biogenesis and autophagy flux during starvation. Collectively, these data supported the conclusion that LDs play a pivotal role in buffering de novo fatty acid synthesis in order to maintain ER homeostasis and intact autophagy regulation.

In addition, performing mass spectrometry-based lipidomics, our analysis revealed significant changes in the relative composition of major phospholipids in a LD-dependent manner. LD-deficient cells display increased phosphatidylinositol (PI) and decreased phosphatidic acid levels. Both, PtdIns (or PI derivatives) and phosphatidic acid are effectors of cellular processes including autophagy and phospholipid biosynthesis. Growing cells in inositol-free media allowed us to metabolically restore the phospholipid composition of LD-deficient cells and to partially improve autophagosome biogenesis and autophagy flux. Importantly, concomitantly restoring phospholipid composition and improving fatty acid resistance fully restores autophagy in the absence of LDs during starvation. In summary, our data indicate that, despite high amounts of stored lipids, LDs are not a required membrane source for the

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formation of autophagosomes. While we do not rule out that LD-derived lipids can contribute to autophagosome biogenesis, our data strongly support a model in which LDs play a fundamental role in controlling phospholipid composition and fatty acid buffering capacity of cells in order to maintain ER homeostasis and intact autophagy regulation.

Our data indicate that conditional dysregulation of the autophagy machinery rather than insufficient membrane supply causes defects in autophagosome formation in the absence of LDs. However, what the underlying mechanisms are and how unbuffered fatty acid synthesis and altered phospholipid composition impinge on the autophagy machinery remain open questions. In this context, determining the nature and origin of the multiple Atg8 puncta detected in LD-deficient cells during starvation will be informative. Their quantitative association with ER exit sites is consistent with multiple events of unproductive autophagosome biogenesis. Alternatively, these structures might represent ER-targeted autophagy in response to fatty acid-induced ER stress during starvation in cells lacking LDs. Consistent with this notion, chemical inhibition of fatty acid synthesis prevents formation of multiple Atg8 puncta in our study.

It is interesting to note that, despite the absence of external fatty acids in our experiments, LD-deficient cells fail to adjust the rate of fatty acid synthesis to a noninhibitory level for autophagy. This observation suggests that regulatory mechanisms controlling fatty acid synthesis evolved inherently depending on the buffering capacity of LDs. Thus, metabolic conditions exceeding the fatty acid buffering capacity of LDs might pose a general problem for cells in regulating autophagy. Interestingly, mammalian model systems of obesity indeed display changes in phospholipid composition linked to autophagy dysregulation. Based on these considerations, the buffering capacity of LDs for fatty acids emerges as a critical determinant for autophagy regulation and cellular stress resistance. However, the underlying regulatory mechanisms remain to be carefully analyzed.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.