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COMMENTARY

TMEM41B functions with VMP1 in autophagosome formation

Keigo Morita, Yutaro Hama, and Noboru Mizushima

Department of Biochemistry and Molecular Biology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

ABSTRACT

Macroautophagy/autophagy requires many autophagy-related (ATG) proteins. Most of the *ATG* genes were identified by genetic screening using simple model organisms. Recently, we performed a forward genetic screen in mammalian cells using the CRISPR-Cas9 system and our autophagic flux reporter GFP-LC3-RFP. One of the identified proteins was TMEM41B, an ER-localized multi-spanning membrane protein. TMEM41B has a characteristic transmembrane domain (the VTT domain), which is also found in VMP1, another protein involved in autophagy. Our results show that TMEM41B and VMP1 are physically and functionally associated.

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Macroautophagy is a complex and dynamic process that requires many molecules. Currently, more than 40 *ATG* genes are known, including some that are required for specific types of autophagy such as pexophagy, mitophagy, reticulophagy, and nucleophagy. Most of these *ATG* genes were identified by genetic screening using model organisms including *Saccharomyces cerevisiae, Komagataella phaffii, Caenorhabditis elegans*, and *Schizosaccharomyces pombe*. RNAi-mediated screening has been performed in mammalian cells, but the recently developed CRISPR-Cas9 system allows knockout (KO)-based genome-wide screening to be performed efficiently. Indeed, DeJesus et al. found that the UFMylation pathway regulates the amount of SQSTM1/p62, a selective substrate of autophagy, and Goodwin et al. discovered an autophagy-independent lysosomal targeting pathway by CRISPR-based genome-wide screening.

Recently, we also performed genome-wide screening to search for novel autophagy regulators in mammals [1]. In this screen, we used GFP-LC3-RFP, an autophagic flux reporter established in our laboratory. This reporter is cleaved into GFP-LC3 and RFP by endogenous ATG4 family proteins. When autophagy is induced, GFP-LC3 is delivered to lysosomes by autophagy and quenched, whereas RFP stays in the cytosol and serves as an internal control. The signals of this reporter can be detected by flow cytometry and used in a 2-dimensional chart, enabling clear separation between normal and autophagy-deficient cell populations (Figure 1(a)). As a result, we succeeded in identifying most nonredundant canonical *ATG* genes (*ATG3, ATG4B, ATG5, ATG7, ATG9A, ATG10, ATG12, ATG13, ATG14, ATG16L1, ATG101*, and *RB1CC1/FIP200*) as well as genes encoding components of the homotypic fusion and protein sorting (HOPS) complex (*VPS16* and *VPS33A*), negative regulators of MTORC1 (*TSC1* and *TSC2*), and other known autophagy regulators (*VPS15, EPG5*, and *EI24*). In addition, *TMEM41B*, a novel *ATG* gene, was identified.

TMEM41B, like VMP1, is a multi-spanning membrane protein that localizes in the endoplasmic reticulum (ER). TMEM41B has a characteristic transmembrane domain in the central region. As similar domains are found in other proteins such as VMP1, yeast Tvp38, and even bacterial proteins, we named it the VTT (VMP1, TMEM41, Tvp38) domain. In *TMEM41B*-KO cells, autophagosome formation is blocked at an early step, as elongated phagophores are not detected. Lipid droplets accumulate in *TMEM41B*-KO cells. The phenotype of *TMEM41B*-KO cells is reminiscent of *VMP1*-KO cells, suggesting a close relationship between TMEM41B and VMP1.

In fact, TMEM41B physically interacts with VMP1 in vivo and in vitro. Moreover, in contrast to overexpression of TMEM41B in *VMP1*-KO cells, overexpression of VMP1 rescues the autophagy deficiency of *TMEM41B*-KO cells. According to classical genetic principles, these results suggest that VMP1 functions downstream of TMEM41B (Figure 1(b)). Thus, TMEM41B may assist the function of VMP1 in phagophore elongation.

At nearly the same time, 2 other groups also identified *TMEM41B* as a novel *ATG* gene. Moretti et al. identified *TMEM41B* in a CRISPR-based screen using endogenous SQSTM1/p62 and CALCOCO2/NDP52 as indicators. The autophagic phenotype that they observed was almost identical to the phenotype that we observed. They further characterized the lipid accumulation phenotype and discovered that mobilization and utilization of fatty acids were delayed in *TMEM41B*-KO cells. Shoemaker et al. also identified *TMEM41B* in a CRISPR-based screen using tfReceptor (RFP-GFP tagged autophagy receptor) probes.

CONTACT Noboru Mizushima nmizu@m.u-tokyo.ac.jp Department of Biochemistry and Molecular Biology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

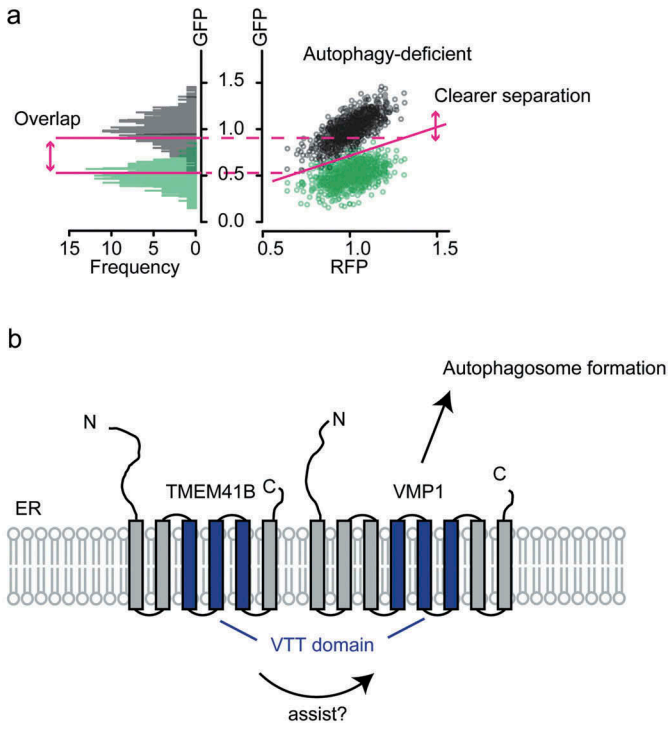

Figure 1. Screening strategy and the structure of TMEM41B and VMP1. (a) Signals from the GFP-LC3-RFP probe can be detected by flow cytometry. A dual-color reporter that yields an internal control (RFP) reflecting the expression level of the reporter allows a clearer separation than a single-color reporter that lacks an internal control. Gray dots indicate autophagy-deficient cells; green dots indicate normal cells. (b) Hypothetical structures of TMEM41B and VMP1. The VTT domain is conserved in both proteins, which form a complex in the ER membrane. TMEM41B and VMP1 cooperate to form autophagosomes.

What are the functions of TMEM41B and VMP1?

To answer this question, we need to know the function of the VTT domain. The VTT domain is widely conserved from bacteria to eukaryotes. *Escherichia coli* YdjX and YdjZ, which have VTT domains, are members of the DedA family. Although still speculative, DedA family proteins are predicted to be half-transporters. If this hypothesis is correct, TMEM41B and VMP1 may also be half-transporters and become full-transporters of ions or lipids when they form homo- or hetero-oligomers. To understand their precise functions, it is necessary to determine their structures and to perform in vitro experiments. Further information will provide new insights into the role of these VTT domain proteins and the ER in autophagosome formation.

Screening limitations

Our CRISPR-based screen was almost saturated, as evidenced by the fact that a nearly complete set of nonredundant *ATG* genes was identified and only *TMEM41B* was novel. However, there were 2 major limitations. First, genes essential for cell survival and growth could not be detected because cells lacking such genes were excluded during the 3 enrichment cycles. Shortening the selection period may have increased the number of surviving clones but would have resulted in a low signal-to-noise ratio. Second, our screening strategy could not identify genes that have functional homologs. Indeed, *ULK1/2, ATG2A/B*, and *WIPI1, WIPI2, WDR45B/WIPI3* and *WDR45/WIPI4* were not detected. This problem could be overcome by using multiplex gRNA libraries. Thus, it is still possible that more novel *ATG* genes exist.

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ORCID

Noboru Mizushima http://orcid.org/0000-0002-6258-6444

Reference

[1] Morita K, Hama Y, Izume T, et al. Genome-wide CRISPR screen identifies TMEM41B as a gene required for autophagosome formation. J Cell Biol. 2018;217(11):3817–3828.