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LC3

Veronica Boggio, Daniel Grasso and Maria Ines Vaccaro

School of Medicine and School of Pharmacy and Biochemistry, University of Buenos Aires, 954 Junin p5, C1113AAD Buenos Aires, Argentina

e-mail: mvaccaro@ffyb.uba.ar

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1. General Function

Autophagy is an evolutionarily conserved degradative process able to recycle cellular components and even whole damaged organelles (1). During this process the targets to be degraded are delivered to a double membrane structure, the autophagosome, which fuses with lysosomes forming the autolysosomes where cargo degradation occurs (2).

The microtubule-associated protein light-chain 1 (MAP1LC3 or LC3) is a small protein of 18 kDa and 125 amino acids (UniProtKB/Swiss-Prot: Q9H492 and Q9GZQ8) involved in autophagosome formation that belongs to the MAP1 LC3 family (3).

Initially, the autophagy process was described at the molecular level in yeast where genes and proteins related to that pathway were named ATG and Atg respectively. Atg8 is a yeast protein associated to the autophagosome membrane, and LC3 together with GABARAP and GATE-16, are its mammalian homologues. LC3 is the most characterized of them and little is known about the roles of GABARAP and GATE-16 in autophagy.

During the autophagy process, LC3 is cleaved and conjugated as part of an Ubiquitin-like conjugation system. During autophagy, Atg4 cleaves the soluble form of LC3 (LC3-I) at the carboxyl-terminal Met-121 to expose Gly-120 (4). Then, the E1-like enzyme Atg7 and the E2-like enzyme Atg3 covalently conjugate LC3 to phosphatidyl-ethanolamine leading to its autophagosome membrane association (LC3-II) (5).

Recently, it has been demonstrated that the polyubiquitin-binding proteins p62/SQSTM1 and Nbr1 (6) interact with polyubiquitinated targets through an UBA domain (Ubiquitin associated domain), and with LC3 through a LIR domain (LC3 interacting region). Therefore LC3 would have a role as an adaptor protein between the autophagosome vesicle and its target to be degraded by lysosomes (7-10). Moreover, LC3 participates directly in the mitochondrial protein Nix recognition during selective autophagic degradation of mitochondria (mitophagy) (11). A similar function has been encountered about PINK1 and Parkin in Parkinson's disease (12).

A recent discovered protein named FYCO1 has been related to microtubule-mediated vesicle transport through its binding to LC3 and PI3P (phosphatidylinositol-3 phosphate) (13).

In conclusion, LC3 is a key molecule in autophagy as a link between the autophagosome and its target cargo, in both selective and non selective autophagy.

2. LC3 in Pancreas

Using a transgenic mouse which constitutively expresses LC3-GFP fusion protein it was possible to evaluate LC3 behavior in the pancreas (14). In acinar cells, few GFP-LC3 dots were present in basal condition. During starvation, autophagosomes and autolysosomes were detected, some of which enclosed zymogen granules. (14).

Proikas-Cezanne et al. also identified LC3 in pancreas, in 2004 (15). They found autophagosomes with co-localization of LC3 and WIPI proteins, which are aberrantly expressed in many human tumors, including pancreatic cancer. Furthermore, in a study retrieved from 71 cases treated by curative pancreaticoduodenectomy, LC3 expression was found by immunocytochemistry in the peripheral area of pancreatic tissue (16).

During chronic pancreatitis in rats, LC3 was found expressed in pancreatic stellate cells after treatment with tocotrienol which induced autophagic and apoptotic stellate cell death by targeting the mitochondrial permeability transition pore (17).

In experimental acute pancreatitis induced by caerulein treatment, autophagy induction with LC3 recruitment and LC3-VMP1 colocalization in autophagosomes were observed in the injured acinar cell cytoplasm (18).

In a recent work involving mice fed with trypsin inhibitor (camostat), pancreas regression was associated with changes in the expression of the autophagosome marker LC3 (19).

Finally, autophagy and LC3-I to LC3-II conversion were described in beta cell during experimental diabetes. VMP1 and LC3 colocalization was also found in beta cells early after streptozotocin-induced diabetes, preceding apoptotic cell death (20). Moreover, palmitate-treated INS-1 beta cells showed autophagosome and autolysosomes formation as well as LC3 recruitment after 12h of 25 mM glucose treatment (21). These results suggest that autophagy may function as an early cellular event in beta cells during experimental diabetes.

3. Tools for Study

The major use of LC3 is as an autophagy marker. Its lipidation and specific recruitment to autophagosomes (LC3-I to LC3-II conversion, describe above) is visualized as a shift from diffuse to punctuate by immunofluorecence or by a fluorescent protein tagged chimera (Figure). Moreover, western blot analysis allows the detection of relative quantities of LC3I (18kD) and LC3 II (16 kD).

a. cDNA clones

Invitrogen provides several LC3 clones, two from human gene (Cat. #3909192; #IOH42908) and two from mouse (Cat. #IOM18103; #IOH10043). OriGene offer a GFP-tagged clone (Cat #RG207356), a Myc-DDK-tagged clone (Cat. #MR200654), and an untagged clone in CMV expression vector (Cat. #SC111851). None of these have been checked by us.

Recently an excellent strategy based on the use of in-tandem tagged LC3 (tfLC3) to evaluate the autophagy flow has emerged (22). During autophagy, autophagosomes move toward the lysosomal organelle, where autophagosome and its cargo are degraded by lysosomal enzymes. In this technique autophagosome-associated LC3 shows a bright yellow fluorescence due to simultaneous excitation of RFP and GFP proteins, which are merged in tandem to LC3 protein structure. Inside of lysosome, RFP is less susceptible to lysosomal enzymes degradation than GFP. Therefore, after fusion between autophagosome and lysosome, autophagosome-associated LC3, only shows the RFP fluorescence. As a result, this method, it is a valuable tool to differentiate autophagosomes from autolysosomes.

b. Antibodies

There are several LC3 antibodies available commercially, although, it is very hard to find a good LC3 antibody. We have used the goat polyclonal antibody raised against an internal region of MAP/LC3 (F14) of human origin from

Santa Cruz Biotechnology (#sc-16756) to identify LC3 in pancreatic tissue and in pancreatic cell lines (AR42J, Panc and MiaPaca) (18). Besides, we often check and validate others LC3 antibodies. There is much variability in antibody performance among brands and even among lots. Therefore, it is recommendable to evaluate different antibody options and they should be validated before use.

c. Viral Vectors

Cell Biolabs Inc. has three GFP-LC3 expression vectors:

- pCMV-GFP-LC3 (CBA-401): a simple mammalian expression vector in which human LC-3B gene is fused in frame with GFP in the expression vector (23).
- pMXs-GFP-LC3 (RTV-801): a retroviral expression vector in which human LC-3B gene is fused in frame with GFP and the GFP-LC3 insert is cloned between BamHI and NotI of pMXs vector (Cat.# RTV-010) (23).
- pSMPUW-GFP-LC3 (LTV-801): a lentiviral expression vector in which human LC-3B gene is fused in frame with GFP and the GFP-LC3 insert is cloned into pSMPUW-Puro vector (Cat.# VPK-212) (23).

d. Mouse Models

Mizushima et al., (14) have generated a transgenic mouse that systemically express GFP-LC3 fusion protein, and serves as a marker for monitoring autophagosomes and LC3 expression in vivo. The authors exposed these animals to starvation in order to detect autophagy. However, in some tissues, autophagy occurs without starvation suggesting that in mammals the role of autophagy is not restricted to starvation.

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Figure legend:

AR42J rat acinar cells transfected with the pRFP-LC3 expression plasmid. Left panel: diffuse distribution of LC3 protein in AR42J cells under standard culture conditions. Right panel: LC3 punctuated pattern is observed in cells under starvation as an autophagy inductor. Fluorescence was observed using a fluorescence microscope Nikon Eclypse 200 (Plan100).