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MERIT, a cellular system coordinating lysosomal repair, removal and replacement

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ABSTRACT

Membrane integrity is essential for cellular survival and function. The spectrum of mechanisms protecting cellular and intracellular membranes is not fully known. Our recent work has uncovered a cellular system termed MERIT for lysosomal *me*mbrane *r*epai*r*, removal and replacement. Specifically, lysosomal membrane damage induces, in succession, ESCRT-dependent membrane repair, macroautophagy/autophagy-dominant removal of damaged lysosomes, and initiation of lysosomal biogenesis via transcriptional programs. The MERIT system is governed by galectins, a family of cytosolically synthesized lectins recognizing β-galactoside glycans. We found in this study that LGALS3 (galectin 3) detects membrane damage by detecting exposed lumenal glycosyl groups, recruits and organizes ESCRT components PDCD6IP/ALIX, CHMP4A, and CHMPB at damaged sites on the lysosomes, and facilitates ESCRT-driven repair of lysosomal membrane. At later stages, LGALS3 cooperates with TRIM16, an autophagy receptor-regulator, to engage autophagy machinery in removal of excessively damaged lysosomes. In the absence of LGALS3, repair and autophagy are less efficient, whereas TFEB nuclear translocation increases to compensate lysosomal deficiency via de novo lysosomal biogenesis. The MERIT system protects endomembrane integrity against a broad spectrum of agents damaging the endolysosomal network including lysosomotropic drugs, *Mycobacterium tuberculosis*, or neurotoxic MAPT/tau.

Abbreviations: AMPK: AMP-activated protein kinase; APEX2: engineered ascorbate peroxidase 2; ATG13: autophagy related 13; ATG16L1: autophagy related 16 like 1; BMMs: bone marrow-derived macrophages; ESCRT: endosomal sorting complexes required for transport; GPN: glycyl-L-phenylalanine 2-naphthylamide; LLOMe: L-leucyl-L-leucine methyl ester; MAP1LC3/LC3: microtubule associated protein 1 light chain 3; MERIT: membrane repair, removal and replacement; MTOR: mechanistic target of rapamycin kinase; TFEB: transcription factor EB; TFRC: transferrin receptor; TRIM16: tripartite motif-containing 16

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Endomembrane damage is a major threat to cellular homeostasis and cell survival. Lysosomes are digestive organelles interacting with endosomal, phagosomal and autophagosomal organelles, providing a range of activities including provision of nutrients, termination of growth factor signaling, and elimination of invading microbes. Lysosomes also play a central role in regulating cellular metabolism and catabolic and anabolic processes via direct effects on MTOR and AMPK. By the nature of their function, lysosomes are exposed to endogenous or exogenous damaging agents including toxic protein aggregates, microbial pathogens, environmental agents such as crystalline silica, endogenous crystals composed of cholesterol or uric acid, and a variety of lysosomotropic drugs, all inducing lysosomal membrane damage. Lysosomal damage leads to several responses including inactivation of MTOR, activation of AMPK signaling, ESCRT membrane repair, autophagy-dominated removal, and TFEB nuclear translocation. How these systems are orchestrated to balance cellular homeostasis is not known.

Certain galectins accumulate on damaged lysosomes and have been long known as lysosomal membrane damage markers. Galectins are a family of cytosolic (as well as extracellularly secreted) proteins binding β-galactoside moieties within glycoproteins and possibly glycolipids. We found that LGALS3 (galectin 3) plays an important role in lysosomal repair [1]. Overexpressing LGALS3 protects lysosomes against damage caused by Leu-Leu-OMe (LLOMe), which induces lysosomal membrane permeabilization, as revealed by lysosomal quality assays including LysoTracker Red staining reflecting lysosomal acidification status, and Magic Red, a reporter of CTSB (cathepsin B) activity in lysosomes. Knocking out *LGALS3* by CRISPR exacerbates lysosomal damage rendering lysosomes more sensitive to LLOMe exposure.

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To further study how LGALS3 safeguards lysosomes, we performed proteomic analyses using proximity biotinylation by APEX2 fused with LGALS3, and liquid chromatography tandem mass spectrometry (LC/MS/MS) and quantitative DIA (EncyclopeDIA/scaffoldDIA) analyses. We found that LGALS3 dynamically associates with parts of the ESCRT components, especially PDCD6IP/ALIX, which in turn recruits ESCRT-III proteins to repair damaged lysosomes. Using biophysical and biochemical approaches including super-resolution microscopy and LysoIP isolation of lysosomes, we showed that LGALS3 and PDCD6IP colocalize and accumulate on damaged lysosomes. The LGALS3-PDCD6IP-ESCRT-III system acts in parallel to the ESCRT-I component TSG101. The association between LGALS3 and PDCD6IP dramatically increases whereas TSG101 departs from LGALS3 during lysosomal damage. LGALS3 is responsible for PDCD6IP recruitment to damaged lysosomes, and this is dependent on LGALS3's ability to bind glycans, which is also key for LGALS3 to recognize damaged lysosomes. Based on proteomic and follow-up functional analyses, we identified a surprising glycosylated protein partner recognized by LGALS3 during lysosomal damage: TFRC (transferrin receptor). TFRC is a glycosylated protein usually associated with early and recycling endosomes, but known to penetrate deeper into the endolysosomal network under stress, and is found in LysoIP-purified lysosomes in starved cells. TFRC is found in immune protein complexes with LGALS3, this interaction increases upon lysosomal damage, and the LGALS3-TFRC association depends on glycan binding capacity of LGALS3. A knockdown of TFRC confirmed that TFRC plays a role in LGALS3 recruitment to damaged lysosomes, albeit additional binding partners contribute including LAMP2, as identified through proteomics. Nevertheless, TFRC dominantly affects PDCD6IP-LGALS3 interactions.

In addition to direct association with LGALS3, PDCD6IP recruitment to damaged lysosomes is affected by another signal, Ca^{2+}. Ca^{2+} and LGALS3-dependent mobilization of PDCD6IP are sequential, separated in time during early stages of lysosomal damage with Ca^{2+} transients acting first and briefly. Downstream of these events, LGALS3 facilitates assembly of other parts of the ESCRT machinery during lysosomal damage by promoting recruitment of ESCRT-III components to damaged lysosomes. The associations between LGALS3 and ESCRT-III components, such as CHMP4A and CHMP4B detected in LGALS3 proteomic assays, are enhanced at LGALS3-dependent stage following LLOMe treatment, e.g. 1 mM LLOMe for 30 min, and the recruitment of CHMP4A and CHMP4B to damaged lysosomes depends on LGALS3. Overexpressing LGALS3 augments the association between PDCD6IP and CHMP4B whereas, conversely, this association decreases in *LGALS3* knockout cells. The regulation of ESCRT-III by LGALS3 depends on LGALS3's glycosyl groups binding ability.

LGALS3 has been implicated in playing a role in autophagy in response to lysosomal injury. In this study, we further confirmed that LGALS3 positively regulates autophagy during removal of damaged lysosomes. Several autophagy markers puncta formation, ATG13, an initiation factor of autophagy, ATG16L1 and LC3, essential elements for autophagosome formation, are affected by LGALS3. The capacity of LGALS3 to recognize glycans is required to initiate autophagy in response to lysosomal damage.

How does LGALS3 coordinate the stages of ESCRT repair and autophagy removal during lysosomal injury? To address this question, we examined the time-dependent association between LGALS3 and TRIM16. TRIM16 interacts with LGALS3, but only once TRIM16 is in protein complexes with a key autophagy initiation regulator, ULK1. We found that TRIM16 is in the complex with LGALS3 only at later stages of lysosomal injury, when ESCRT components, PDCD6IP and CHMP4A, depart from LGALS3. Interactions between LGALS3 and ESCRT are affected by TRIM16, and, vice versa, ESCRTs affect LGALS3 and TRIM16 interactions. In conclusion, LGALS3 functions as a switch from ESCRT membrane repair to autophagic removal during lysosomal damage. Lastly, in *LGALS3* KO cells, where ESCRT repair and autophagy removal are inefficient, this hyperactivates the lysosomal replacement system executed by TFEB, as the last stage for the MERIT system.

Our study also places elements of the MERIT system in the physiological context of cellular invasion by *Mycobacterium tuberculosis* and demonstrates its protective value against this pathogen both ex vivo in primary

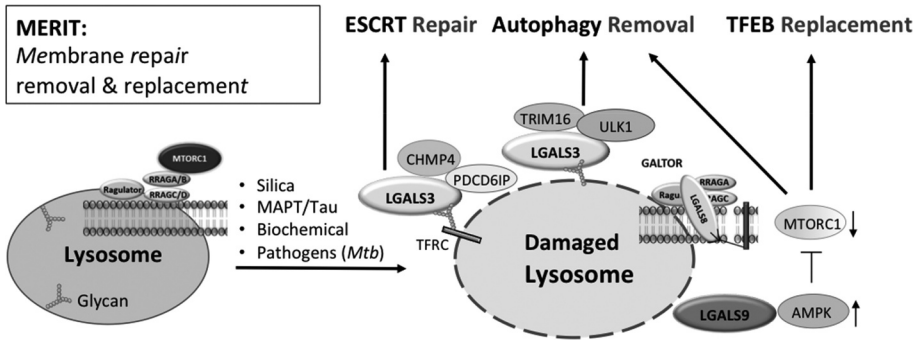

Figure 1. Model depicting the MERIT system and how it coordinates lysosomal membrane repair, removal and replacement. Lysosomes are damaged under certain conditions, inducing the activation of the MERIT system: LGALS3 recruits ESCRT components PDCD6IP and CHMP4A/B and promotes the formation of ESCRT machinery to repair damaged lysosomes. LGALS3 cooperates with the autophagic receptor-regulator TRIM16 to guide autophagy initiation machinery to remove injured lysosomes. LGALS8 inactivates MTOR during lysosomal damage. LGALS9 activates AMPK upon lysosomal injury. AMPK further inhibits MTOR. The MTOR inactivation and AMPK activation lead to induction of autophagy and TFEB nuclear translocation to replace damaged lysosomes through de novo biogenesis.

macrophages from *trim16*$^{Fl/Fl}$ *Lyz2/LysM-Cre* mice as well as in vivo in the murine model of tuberculosis with aerosol infection of *trim16*$^{Fl/Fl}$ *Lyz2/LysM-Cre* and their Cre^{-} littermates. In addition to microbial invasion, we also tested in cell culture other lysosomal damage conditions, such as exposure to silica crystals, lysosome-damaging dipeptide GPN, and a neurotoxic form of MAPT/tau.

In conclusion, the MERIT system represents a system (Figure 1) of escalating homeostatic responses launching membrane repair first via ESCRTs, deploying removal through autophagy as needed, and initiating the replacement program through de novo biogenesis via TFEB, all regulated and coordinated by galectins. LGALS3 coordinates ESCRT membrane repair and autophagic removal. Other galectins control MTOR (LGALS8) and AMPK (LGALS9), leading to autophagy response and TFEB-mediated lysosomal replacement as shown elsewhere. We postulate that the MERIT system, along with its components taken individually, plays a significant role in infection, inflammation, neurodegeneration, metabolic disorders and cancer.

Disclosure statement

No conflicts of interest.

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Reference

[1] Jia J, Claude-Taupin A, Gu Y, et al. Galectin-3 coordinates a cellular system for lysosomal repair and removal. Dev Cell. 2020;52:69–87.e8. PMID: 31813797.