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Research Highlight

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Receptor-mediated reticulophagy: a novel promising therapy target for diseases

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Autophagy is a highly conserved self-digestion process raging from lower eukaryotes to mammals. Autophagy involves in the degradation of misfolded protein aggregates and damaged organelles, which are subsequently reused. Upon autophagy is initiated, a membrane structure termed the phagophore, the precursor of autophagosome, gradually expands and engulfs misfolded protein or damaged organelles and delivers them to the vacuole/lysosome for degradation. Autophagy contributes to the process of survival and death. Basic autophagy is essential for maintaining cellular homeostasis. During normal physiology, specialized cellular function requires the regulation of autophagy by scavenging misfolded protein or damaged organelles. However, excessive and dysregulated autophagy may induce apoptosis and even cell death due to enzymes leaking from lysosomes [1].

Autophagy consists of three categories: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy is known as a most active and extensive form, which can degrade intracellular misfolded protein or damaged organelles. Several autophagy (Atg)-related proteins are recruited to participate in this process. Compared with macroautophagy, microautophagy can occur in absence of functional core autophagy machinery. The main character of microautophagy is that cytoplasmic cargos are engulfed into lysosomes directly and inward budding of the lysosomal membrane. Chaperone-mediated autophagy is closely associated with the selective degradation of proteins with a specific-signal sequence called the 'KFERQ' motif and requires the participation of the molecular chaperone Hsc70 [2].

Recently, the phenomenon of selective degradation of organelles, called selective autophagy, has been shown. Selective autophagies include mitophagy, reticulophagy, ribophagy, pexophagy, crinophagy, heterophagy, xenophagy, aggrephagy, and lipophagy. Furthermore, some receptor proteins, which mediate selective autophagy, have been identified. There are several identified receptor proteins including the autophagy receptors p62, Nbr1, Nix, NDP52, Smurf1/optineurin, and c-Cbl. These receptor proteins are characterized by the presence of the LC3-interacting region (LIR), which interact with the autophagosome membrane bound LC3 family members LC3/GABARAP/GATE-16 [3,4].

The endoplasmic reticulum (ER), a very complex and elaborate cellular organelle, is composed of rough ER and smooth ER. The ER contributes to the folding and delivery of proteins by the secretory pathway. The accumulation of misfolded proteins in ER triggers the process of autophagy in cells. ER itself also can be subject to autophagy, which is reticulophagy. Accumulation of aggregation-prone proteins in the ER appears to play a critical role in removing ER fragments via reticulophagy [5]. It has been reported that short ER fragments are present in starvation-induced autophagosomes in yeast [5].

In the process of reticulophagy, receptor proteins may play a central role in target selection. Mochida *et al.* [6] recently revealed that the ER in yeast cells was degraded via selective autophagy. A novel Atg8-binding protein Atg40 in the budding yeast *Saccharomyces cerevisiae* was identified. Atg40 was turned out to be the receptor, which mediates the process of reticulophagy. In *S. cerevisiae*, the ER contains three subdomains, the cytoplasmic ER (cytoER), the cortical ER (cER), and the perinuclear ER (pnER). Atg40 was mainly localized in the cytoER and cER. A noticeable decrease of Atg40 expression has been observed under nutrient-replete conditions, while the expression of Atg40 was increased by treating with rapamycin. Atg40 can interact with Atg8 and facilitate the formation of autophagosomes. Overexpression of Atg40 stimulated reticulophagy by loading fragments of cytoER and cER subdomains into the phagophore. Knockout of *Atg40* was found to inhibit reticulophagy and reduced the degradation of cytoER and cER. So, Mochida *et al.* [6,7] provided evidence supporting that the cytoER and cER are degraded by Atg40 receptor-mediated selective autophagy in *S. cerevisiae* (**Fig. 1A**).

Atg40 is probably the functional counterpart of family with sequence similarity 134 member B (FAM134B), an autophagy receptor for the reticulophagy in mammals. FAM134B, a member of the

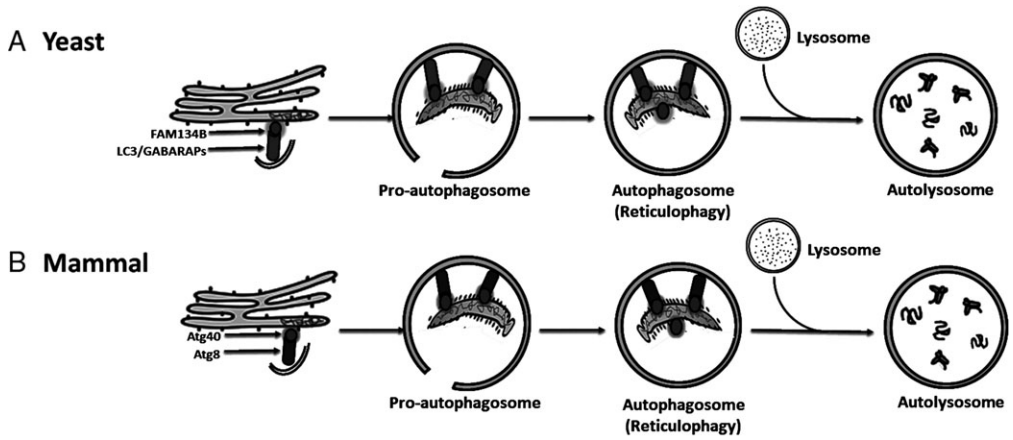

Figure 1. The process of receptor-mediated reticulophagy (A) In the yeast *S. cerevisiae*, Atg40 is enriched in the cortical and cytoER, and loads these ER subdomains into autophagosomes. (B) FAM134B reticulon protein, an autophagy receptor for the ER in mammals, is an ER-resident receptor that binds to autophagy modifiers LC3 and GABARAP, and facilitates the process of reticulophagy.

FAM134 reticulon proteins, is anchored in the cytoER network. Khaminets *et al.* [8] revealed that ER-resident receptor FAM134B contains a conserved putative LIR (motif). FAM134B-positive ER fragments co-localize with LC3B, suggesting that FAM134 binds to autophagy modifiers LC3 and GABARAP. Down-regulation of FAM134B resulted in a striking swelling of the ER, while overexpression of FAM134B led to ER fragmentation and lysosomal degradation. These results indicated that FAM134B, an ER-selective autophagy receptor, facilitates ER degradation by reticulophagy. *In vivo*, disruption of FAM134B in mice led to swelling of the ER and inhibited reticulophagy. Therefore, they revealed that FAM134B is the autophagy receptor, which mediates the process of reticulophagy in mammal (**Fig. 1B**).

Together, these observations demonstrated that Atg40 and FAM134B are the important receptors that mediate the process of reticulophagy by core autophagy machinery. Conversely, Schuck *et al.* [5] reported that reticulophagy also occurred independent of core autophagy machinery in yeast. The precise regulation and mechanisms of receptor-mediated reticulophagy still need to be further explored. Moreover, attention should also be paid to how the cell regulates the segregation of the unwanted parts of the ER and how this breaks away from the organelle. Lipatova and Segev [9] have revealed that reticulophagy may be important to maintain ER homeostasis. Autophagy may play a protective role against ER stress-induced cell death, as autophagy-deficient cells show higher vulnerability to ER stress, and conversely, pretreatment with rapamycin makes cells more resistant to this damage. However, how reticulophagy contributes to cell homeostasis and cell fate is still not clear. The development of specific inhibitors and/or activators of reticulophagy is pivotal in future research. Thus, the research of reticulophagy is an attractive field to be explored [10].

A few methods have been developed to study receptor-mediated reticulophagy. Electron microscopy can be used to morphologically characterize the ER, which is sequestered into the autophagosome. Fluorescence microscopy can be used to demonstrate the ER fragments co-localized with LC3B or CD63-positive lysosomes. Yeast two-hybrid assays and immunoprecipitation analysis can be used to confirm the interactions of reticulon proteins and autophagy marker proteins. Reticulophagy can also be determined by flow cytometry using ER Tracker probes [11]. Furthermore, transgenic mice are effective tools for studying reticulophagy *in vivo*.

Various stressors, such as starvation, hypoxia, infection, inflammatory stimuli, oxidative stress, and drugs, result in ER stress, which may facilitate the process of reticulophagy. ER stress participates in several pathophysiological events such as tumorigenesis, neurodegenerative diseases, cardiovascular disease, fatty liver, diabetes, defenses against intracellular pathogens, antigen presentation, and longevity. So, it is speculated that reticulophagy, which contributes to the protein quality control process in the ER, may also be deeply associated with these diseases. For example, targeting the reticulophagy system in the trabecular meshwork plays an important role in the treatment of glaucoma [12]. *Candida albicans* autophagy alleviates ER stress and is involved in tolerance to antifungal drugs. Under ER stress-related conditions, the fungal cells have evolved multifaceted systems in response to ER stress and reticulophagy. Reticulophagy contributes to the maintenance of ER homeostasis by degrading dysfunctional ER [13]. These findings suggest that reticulophagy may be a novel promising therapy target for diseases and these receptor proteins, such as Atg40 and FAM134B, may be promising candidates for drug screening.

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