News Release

Title

Degradation of mutant protein aggregates within the endoplasmic reticulum of vasopressin neurons

Key Points

- **Endoplasmic reticulum (ER) stress caused by accumulation of misfolded and unfolded proteins has been implicated in a wide spectrum of disorders, including neurodegenerative diseases and diabetes mellitus.**
- **Misfolded or unfolded proteins accumulated in the ER are said to be degraded through the ubiquitin-proteasome system or ER-phagy, in which aggregates in the ER are degraded only after translocation or isolation from the ER.**
- **In this study, we found a novel mechanism by which mutant protein aggregates undergo autophagic-lysosomal degradation within specialized compartments of the ER, without isolation or translocation from the ER.**

Summary

The endoplasmic reticulum (ER) is an organelle mainly responsible for the synthesis, folding, assembly, and transport of proteins. Misfolded or unfolded proteins accumulate in the ER causing ER stress. There are two known mechanisms by which misfolded proteins in the ER are degraded: ER-associated degradation (ERAD) where aggregates are translocated from the ER to the cytosol and degraded through the ubiquitin-proteasome system, and ER-phagy in which a portion of ER containing the protein aggregates is isolated from the remaining ER and degraded by lysosomes. In either case of ERAD or ER-phagy, aggregates in the ER are degraded only after translocation or isolation from the ER. Here, we describe a mechanism by which mutant proteins are degraded within the ER. Aggregates of mutant arginine vasopressin (AVP) precursor were confined to ER-associated compartments (ERACs) connected to the ER in AVP neurons of a mouse model of familial neurohypophysial diabetes insipidus. The ERACs were enclosed by membranes, an ER chaperone and marker protein of phagophores and autophagosomes were expressed around the aggregates, and lysosomes fused with the ERACs. Moreover, lysosome-related molecules were present within the ERACs, and aggregate degradation within the ERACs was dependent on autophagic-lysosomal activity. Thus, we demonstrate that protein aggregates can be degraded by autophagic-lysosomal machinery within specialized compartments of the ER.

Research Background

Familial neurohypophysial diabetes insipidus (FNDI) is an autosomal dominant disease caused by mutations in the AVP gene locus, predominantly in the region encoding neurophysin II (NPII). We previously generated FNDI model mice by introducing a NPII mutation (that causes FNDI in humans) into the AVP gene locus, and the resulting heterozygous mice recapitulated the phenotypes

of patients with FNDI. Electron microscopic analyses of AVP neurons in FNDI mice revealed that aggregates were confined to a specific compartment of the rough ER, termed the ERAC (ER-associated compartment). However, it remains to be elucidated whether ERACs are connected to the intact ER lumen or if there are any mechanisms by which aggregates are degraded within the ERACs.

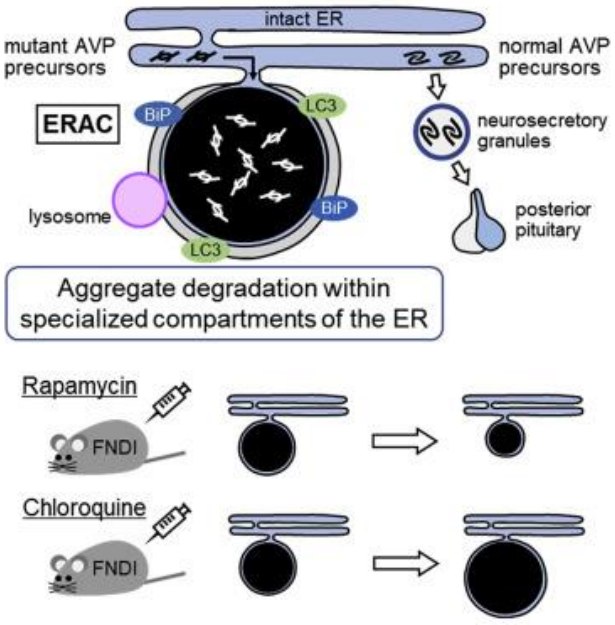
In the present study, we hypothesized that aggregates are degraded by lysosomes within the ERACs which maintain connection to the intact ER lumen. To test this hypothesis, we investigated the following: (1) the structural relationships between ERACs, ER membranes, and lysosomes by serial block-face scanning electron microscopy (SBF-SEM), (2) the localization of several molecules involved in autophagic-lysosomal degradation as well as ER chaperones by immunoelectron microscopy, and (3) the pharmacological effects of inducing or inhibiting the autophagic-lysosomal degradation system on ERAC formation in AVP neurons of FNDI mice.

Research Results

In the present study, SBF-SEM analyses revealed that ERACs, in which mutant NPII was accumulated, were connected to the intact ER and lysosomes were fused to the ERACs via ERAC protrusions. We also showed that ER chaperone BiP and LC3 (marker protein of phagophores and autophagosomes) were expressed around the aggregates, and that lysosome-related molecules LAMP2 and cathepsin D were present within the ERACs. Furthermore, our data showed that the number of ERACs was decreased or increased by rapamycin or chloroquine treatment, respectively.

Research Summary and Future Perspective

Our data demonstrate that mutant proteins undergo autophagic-lysosomal degradation within ERACs, without isolation or translocation from the ER, in AVP neurons of FNDI mice. On the other hand, it is unclear from this study how aggregates and lysosomal acid hydrolases are confined to the ERACs, although further studies are required to clarify the underlying mechanisms.



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