Understanding the Role of Lipid Modifications in ER Protein Quality Control

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Abstract
Endoplasmic reticulum (ER) associated degradation (ERAD) is a cellular process responsible for the identification and degradation of misfolded proteins. Defining the mechanisms underlying this process is vital to understanding the pathogenesis of numerous human diseases that result from impaired ER protein quality control. Recent reports indicate that the inhibition of acyl-CoA synthetases with small molecule, triacsin c disrupts ERAD. However, it remains unknown why acyl-CoA synthetases are required for ERAD. Palmitoylation, the covalent addition of the fatty acid palmitate to proteins, requires a unique acyl-CoA synthetase activity and significantly impacts protein localization, structure, and physical interactions. Therefore, we hypothesized that palmitoylation of ERAD machinery regulates the identification and degradation of ERAD substrates. To test this hypothesis, we employed copper-catalyzed azide-alkyne cycloaddition to probe for palmitoylated ERAD proteins. Our results reveal palmitoylation of four prominent ERAD proteins: the E2-recruitment factor AUP1, the E3 ligase Hrd1, the rhomboid pseudoprotease Derlin 1, and the mannosidase ERMan1, indicating that palmitoylation may regulate ERAD at multiple steps. Consistent with a functional role for palmitoylation in ERAD, we find that the palmitoylation inhibitor 2-BP impairs significantly attenuated ERAD. Together, our results identify an unprecedented mechanism of ERAD regulation that will broadly impact our understanding of ERAD-associated diseases.

Introduction

Figure 1. Schematic of the pathways affected by triacsin c. Triacsin c inhibits long chain acyl-CoA synthetases (ACSLs), which are required for the activation of fatty acids that are employed for protein acylation, β-oxidation, and lipid biosynthesis.

Figure 2. The long chain acyl-CoA synthetase inhibitor triacsin c impairs CD147 degradation. A) CD147 degradation kinetics were analyzed by immunoblotting following a 16-hr triacsin c pretreatment B) CD147 levels in panel (A) were quantified using ImageJ and plotted.

Figure 3. Triacsin c impairs glycan trimming. Immunoblot analysis of CD147 degradation in cells pretreated for 16 hr. with triacsin c or contrasted with the mannosidase inhibitor kifunensine.

Figure 4. Schematic of the click chemistry method used to detect palmitoylation. A) Cells were pretreated for 8 hr. with 17-octadecynoic acid (17-OcDA), an alkyn-containing palmitate analog. B) Affinity purified S-tagged proteins were reacted with the fluorescent azide-rhodamine reporter via copper-catalyzed azide-alkyne cycloaddition.

Methods and Results

Figure 5. Identification of palmitoylated ERAD proteins. A-F) Affinity purified proteins from cells incubated in the presence or absence of 17-OcDA were reacted with a rhodamine moiety to enable detection of palmitoylation. Complexes were also incubated with NaOH to remove the Ser-GSH-linked palmitate group and confirm specificity of the signal. Proteins were separated by SDS-PAGE and gels scanned for fluorescence to detect the reporter or analyzed by Western blotting to detect the affinity purified protein. DFP is a non-fluorescent GFP variant used as a negative control, and calnexin is used as a positive control.

Future Directions

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