Down-Regulation of TRIM11 in Lysosomal Regulation: Investigating its Impact on Cellular Degradation Pathways

Hecheng Xu

ABSTRACT:

Alzheimer's disease is characterized by the accumulation of Aβ plaques and tau protein tangles, with TRIM11 playing a crucial role in tau regulation [1]. However, TRIM11's degradation in Alzheimer's remains unclear. This study investigates whether lysosomes, responsible for cellular degradation, are involved in TRIM11 degradation [2]. Lysosome inhibitors were applied, and TRIM11 levels were examined. Immunostaining for TRIM11 and lysosome inhibitors was conducted to assess co-localization, indicating potential interaction. The findings suggest a connection between TRIM11 and lysosomes in Alzheimer's pathology, warranting further research for potential therapeutic implications [3].

Keywords: Alzheimer's disease, TRIM11, lysosomal regulation."

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder. Amyloid-β (Aβ), which comes from amyloid precursor protein (APP), builds up in brain plaques, and neurofibrillary tangles (NFTs) made of messed-up tau protein are the two main reasons that cause Alzheimer's disease. TRIM11 has a big role in regulating tau protein by controlling its levels, preventing misfolding, and breaking down tau aggregates in Alzheimer's disease. And TRIM11 degrades in Alzheimer's disease. Downregulation of TRIM11 may occur in a stage of mRNA (Transcription), lysosomal regulation (Translation), and proteasomal regulation (Translation) stage [4][5]. To determine whether lysosomes cause TRIM11's degradation, we hypothesize that TRIM11 degrades because lysosomes degrade proteins. Then, we add lysosome inhibitors to block activity and observe if TRIM11 levels increase. Later, immunostaining for TRIM11 and lysosome inhibitors will determine if they interact in the same locations. If they do, it shows that TRIM11 reacts with lysosomes. Utilize confocal microscopy to identify sites of action.

2. PREVIOUS RESEARCH

The TRIM family includes genes that make proteins with a special structure called the tripartite motif. This motif has three parts: a RING domain, a B-box domain, and a coiled-coil region. The RING domain is a part that helps the protein interact with other molecules in the cell. It's important for the protein's job in the cell, which is called E3 ligase activity. The B-box domains are also parts that bind to molecules in the cell. The tripartite motif has a specific order and spacing of these parts. The TRIM family represents the biggest group of RING-containing E3 ubiquitin ligases. The tripartite motif structure has been kept over time and has grown with new TRIM genes. Many of these proteins are involved in changing other proteins in the cell. The RING domain helps with this process called ubiquitination. There are two types of E3 ligases: one with a HECT domain and one with a RING domain. The RING domain helps the proteins come together. The coiled-coil part of TRIM proteins is made of pieces that stick together, strengthening the protein. This part helps the protein interact with others and form groups [6].

Tauopathies are Tau protein mutations and accumulations, leadingad to other neurodegenerative diseases. TRIM11 plays a big role in tau protein in three ways. First, it helps control the amount of mutant tau. It does this by sticking to tau, especially the changed types or those with too many phosphates, and helping them change shape a little. This makes them break down using a part of the cell called the proteasome. This makes TRIM11 a very important connection between tau and the proteasome. Second, TRIM11 helps tau by stopping it from folding up wrong and getting all clumped together. Third, TRIM11 breaks apart tau deposits, even the tough ones, that have already formed. TRIM11 doesn't need a lot of energy, and they work even when there's not much TRIM11 compared to tau.

Lysosomes are involved in macroautophagy, chaperone-mediated autophagy (CMA), and microautophagy. According to the mechanism by which intracellular components enter the lysosome for degradation, autophagy can be categorized into microautophagy, molecular chaperone-mediated autophagy, and macroautophagy [7][8]. In microautophagy, cytoplasmic material is taken up

into lysosomes. Chaperone-mediated autophagy promotes the degradation of cytoplasmic proteins by directly targeting them to lysosomes and entering the lysosomal lumen [9][10][11]. In macroautophagy, cytoplasmic contents subject to degradation are enclosed within double-membrane subcellular structures.

The main difference between the different types is how the lysosome captures the substrate [12]. Macroautophagy is characterized by forming intermediate double-membrane structures, including autophagosomes and autolysosomes [13]. Vesicles engulf cargo and then degraded by fusion with lysosomes [14][15][16]. Chaperone-mediated autophagy selectively recognizes substrate proteins containing KFERQ motifs. Cargo is transported to the chaperonin-mediated lysosome in HSPA8, associated co-chaperones, and the lysosomal membrane protein LAMP2A, rather than establishing any sealed vesicles [17].

LAMP-2A is a glycoprotein found in lysosomal membranes and is a one-way type 1 membrane protein. It has an N-terminal conserved N-glycosylated luminal structural domain, a transmembrane structural domain, and a C-terminal cytoplasmic structural domain. LAMP-2A maintains stability during cytosolic enzyme-mediated autophagy (CMA) by interacting with lysosomal chaperones [18][19][20][21]. The transmembrane structural domain plays a key role in substrate binding
.

Two classes of proteins play a big role in lysosomal function: soluble lysosomal hydrolases (Acid hydrolases) and integral lysosomal membrane proteins (LMPs). Integral lysosomal membrane proteins are present in the lysosomal limiting membrane. LMPs include lysosome-associated membrane protein 1 (LAMP1), LAMP2, lysosomal integral membrane protein 2 (LIMP2; SCARB2), and the four-transmembrane protein CD63. LAMPs are type 1 transmembrane proteins and contain a large, heavily glycosylated luminal domain and a short cytoplasmic tail.

LAMP-2 proteins are found in lysosomes and are intact membrane proteins with two conserved luminal structural domains (constituting 90% of the entire protein), a transmembrane (TM) structural domain (~20 amino acids), and a short structural domain (10-12 amino acids) C-terminal cytoplasmic tail. Earlier studies have also shown that LAMP-2A is a homodimer that forms higher oligomeric complexes with molecular chaperones upon activation of CMA. The formation of this higher oligomer is required for substrate translocation across the lysosomal membrane.

It is essential to explore how it might contribute to the pathogenesis of AD. This research proposal aims to verify if lysosomes down-regulate TRIM11 protein in Alzheimer's disease.

This study's theoretical framework examines the relationship between TRIM11's down-regulation and lysosome. To test this, use lysosomal inhibitors to block lysosomal activities and observe whether TRIM11's levels increase. Additionally, immunostaining techniques for TRIM11 and lysosome inhibitors should be used to establish whether they co-locate. The presence of co-localization would indicate an interaction between TRIM11 and lysosomes.

3. METHODS

Cells were cultured in a suitable medium to achieve optimal cell density. The cells were divided into two groups: the experimental group and the control group. A lysosomal inhibitor (Ammonium chloride) was added to the experimental group and knocked down the LAMP-2a [22]. The control group did not add lysosomal inhibitors (Ammonium chloride). Cells were lysed using a cell lysis buffer containing lysosomal inhibitor (Ammonium chloride), and the proteins were extracted. The specific TRIM11 antibodies were combined with magnetic beads to form an immune complex. Lysed cell extracts were incubated with these immune complexes to isolate TRIM11 and its interacting proteins [23]. Then, wash the immune complexes to remove any non-specifically bound proteins. Use Western blot analysis to separate the sample on an SDS-PAGE gel and transfer it to a membrane. Use Immunoblot analysis of the TRIM11 antibodies and other antibodies related to TRIM11. This analysis helps to determine whether lysosomes cause TRIM11's degradation. The data from these experiments can provide evidence of the involvement of lysosomes in TRIM11 degradation. If we observed, the levels of TRIM11 kept constant when the lysosomes were inhibited. It strongly suggests that lysosomes are responsible for degrading TRIM11. Then, co-localization and immunofluorescence can also support it by TRIM11 and lysosomal markers.

4. CONCLUSION

Our study explored how TRIM11 and lysosomes connect in Alzheimer's disease. Lysosomes might affect TRIM11. This discovery could be vital for treating Alzheimer's. It may help discover the specific molecular pathways and cellular mechanisms that lysosomes degrade TRIM11. The limitation of the study is that it only understands whether lysosomes, responsible for cellular degradation, are involved in TRIM11 degradation. However, more research is needed to focus on the mechanisms that lysosomes degrease TRIM11 in Alzheimer's disease to

fully understand this connection and its potential for improving Alzheimer's treatments.

REFERENCES

[1] "The amyloid hypothesis of Alzheimer's disease at 25 years." *Embo Press*, www.embopress.org/doi/full/10.15252/emmm.201606210.

[2] "TRIM proteins in autophagy: selective sensors in cell damage and innate immune responses." *Nature*, www.nature.com/articles/s41418-020-0495-2.

[3] "Endosomal-lysosomal dysfunctions in Alzheimer's disease: Pathogenesis and therapeutic interventions." *Springerlink*, link.springer.com/article/10.1007/s11011-021-00737-0.

[4] "Principles of lysosomal membrane degradation: Cellular topology and biochemistry of lysosomal lipid degradation." *ScienceDirect*, www.sciencedirect.com/science/article/pii/S0167488908003455.

[5] "Signals from the lysosome: a control center for cellular clearance and energy metabolism." *Nature*, www.nature.com/articles/nrm3565.

[6] "Precision autophagy directed by receptor regulators – emerging examples within the TRIM family." *Journal.biologist.com*, journals.biologists.com/jcs/article/129/5/881/55975/Precision-autophagy-directed-by-receptor.

[7] "LYECs: lysosome-enhancing compounds as potential therapeutic approaches for Alzheimer disease." *Tandfonline*, www.tandfonline.com/doi/full/10.1080/15548627.2022.2131247.

[8] "Lysosomal Network Proteins as Potential Novel CSF Biomarkers for Alzheimer's Disease." *Springerlink*, link.springer.com/article/10.1007/s12017-013-8269-3.

[9] "Lysosome-associated membrane protein 1 (LAMP-1) in Alzheimer's disease." *Onlinelibrary*, onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2990.2006.00756.x.

[10] "Lysosome biogenesis: Regulation and functions." *NIH*, www.ncbi.nlm.nih.gov/pmc/articles/PMC8105738/.

[11] "Lysosome biology in autophagy." *Nature*, www.nature.com/articles/s41421-020-0141-7.

[12] "The lysosome: from waste bag to potential therapeutic target." *Academic. ocp*, academic.oup.com/jmcb/article/5/4/214/900529.

[13] "Lysosome membrane lipid microdomains: novel regulators of chaperone-mediated autophagy." *Embopress.org*, www.embopress.org/doi/full/10.1038/sj.emboj.7601283.

[14] "An Overview of the Molecular Mechanism of Autophagy." *Springerlink*, link.springer.com/chapter/10.1007/978-3-642-00302-8_1.

[15] "Protein misfolding disorders and macroautophagy." *ScienceDirect*, www.sciencedirect.com/science/article/pii/S095506741000181X.

[16] "Regulation of autophagy by lysosomal positioning." *Tandfonline*, www.tandfonline.com/doi/full/10.4161/auto.7.8.15862.

[17] "Revisiting LAMP1 as a marker for degradative autophagy-lysosomal organelles in the nervous system." *Tandfonline*, www.tandfonline.com/doi/full/10.1080/15548627.2018.1482147.

[18] "Arrested maturation of Neisseria-containing phagosomes in the absence of the lysosome-associated membrane proteins, LAMP-1 and LAMP-2." *Wiley*, onlinelibrary.wiley.com/doi/full/10.1111/j.1462-5822.2007.00946.x.

[19] "Autophagic Pathways to Clear the Tau Aggregates in Alzheimer's Disease." *Springerlink*, link.springer.com/article/10.1007/s10571-020-00897-0.

[20] "Autophagy and Alzheimer's Disease: From Molecular Mechanisms to Therapeutic Implications." *Frontier*, www.frontiersin.org/articles/10.3389/fnagi.2018.00004.

[22] "Mechanisms controlling selective elimination of damaged lysosomes." *ScienceDirect*, www.sciencedirect.com/science/article/pii/S2468867322001080.

[23] "Crystal structure of the conserved domain of the DC lysosomal associated membrane protein: implications for the lysosomal glycocalyx." *Biomedcentral*, bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-10-62.

[24] "Hsp90 regulates autophagy and plays a role in cancer therapy." *Springerlink*, link.springer.com/article/10.1007/s13277-015-4142-3.

[25] "Tau degradation: The ubiquitin–proteasome system versus the autophagy-lysosome system." *Nature*, www.sciencedirect.com/science/article/abs/pii/S0301008213000154.