Secretory Carrier Membrane Protein 3's (SCAMP3) Role in β-Amyloid Production and Secretion

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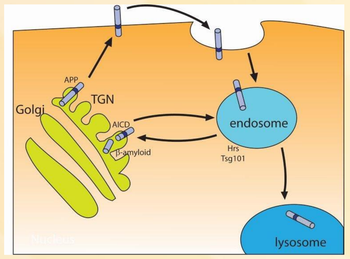
Abstract

Alzheimer's disease is a neurodegenerative disease associated with loss of memory and cognitive function. The aggregation of extracellular plaques containing β-amyloid is related to the processing of the amyloid precursor protein (APP). The degradation of APP is regulated by the endosomal sorting complexes required for transport (ESCRTs) and disruption of ESCRT function leads to accumulation of β-amyloid. Previous studies have shown that secretory carrier membrane protein 3 (SCAMP3) interacts with ESCRTs that function in APP processing. We hypothesize then that SCAMP3 functions in trafficking of APP. We will test this hypothesis by examining the effects of RNA interference of SCAMP3 in two assays: (1) an immunofluorescence colocalization assay with full length APP and (2) and ELISA assay to measure β-amyloid levels. Both assays will use H4 neuroglioma cells that have been stably transfected with APP-EGFP.

Introduction

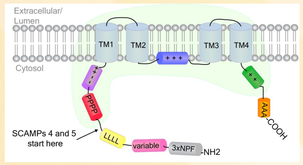
Production of β-amyloid is regulated by membrane trafficking

Alzheimer's disease is an irreversible, progressive neurodegenerative disorder that slowly destroys memory and thinking skills and, eventually, the ability to carry out the simplest tasks. Experts suggest that more than 5 million Americans have Alzheimer's disease. Alzheimer's Disease is caused by the aggregation of extracellular plaques containing β-amyloid which is related to the processing of the amyloid precursor protein (APP) [3]. The degradation of APP is regulated by the endosomal sorting complexes required for transport (ESCRTs) and disruption of ESCRT function leads to transport of APP to the TGN where it is cleaved to β-amyloid. This results in accumulation of β-amyloid [3]. Previous studies have shown that secretory carrier membrane protein 3 (SCAMP3) interacts with ESCRTs that function in APP processing [1].

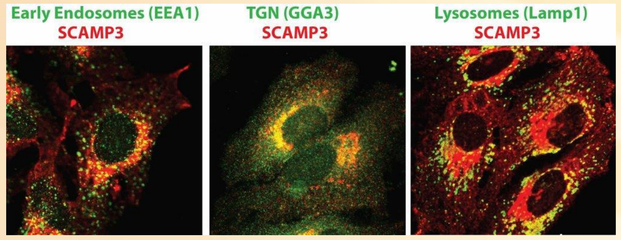


SCAMPS are transmembrane proteins that regulate membrane traffic

The SCAMP family consists of five isoforms , SCAMPs 1-5. SCAMPs have four transmembrane domains with cytoplasmic N- and C-termini that facilitate interactions with other proteins.



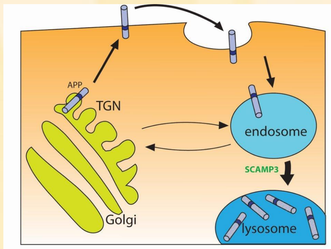
SCAMPs are found in many places including the TGN, endosomes, and plasma membranes [1]. They function in the recycling and degradative process of cell surface receptors [1,2]. SCAMP3 specifically has been shown to regulate degradation of the epidermal growth factor receptor by interacting with and opposing the function of the ESCRT proteins Hrs and Tsg101 [1]. Because SCAMP3 is ubiquitously expressed in many cell types including neurons, it likely plays a role in regulating the trafficking of many other proteins [1,2].



Hypothesis

We hypothesize that SCAMP3 regulates trafficking of APP at the early endosome. We propose that SCAMP3 could perform this function by either (A) promoting trafficking of APP to the lysosome, thus increasing β-amyloid production or (B) inhibiting trafficking of APP to the lysosome, thus decreasing β-amyloid production. Therefore, the knock-down of SCAMP3 should result in colocalization of APP with either the lysosomes or the early endosomes.

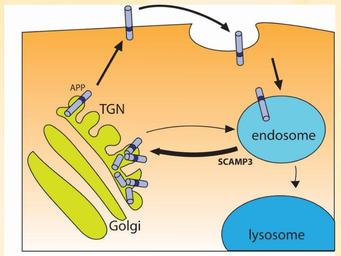
(A) SCAMP3 promotes APP trafficking to lysosome



Then, knockdown of SCAMP3 will:

↓ trafficking of APP to lysosome
↑ β-amyloid production

(B) SCAMP3 inhibits APP trafficking to lysosome



Then, knockdown of SCAMP3 will:

↑ trafficking of APP to lysosome
↓ β-amyloid production

Experimental Design

Immunofluorescence:

Knockdown SCAMP3

H4 cells are transfected with SCAMP3–specific or control siRNA for two days to knockdown SCAMP3 expression

Transfect APP

The control and SCAMP3 knockdown cells are transfected with a plasmid to express Amyloid Precursor Protein, which will be tagged with GFP

Transfect siRNA

24 hours after APP transfection, the cells are transfected with siRNA to knock down SCAMP3 expression

Examine Localization

EEA1 or LAMP1/2 are tagged with red fluorophore, then imaged. These images are superimposed onto images showing APP-GFP expression and analyzed to determine colocalization

ELISA:

Knockdown SCAMP3

The control and SCAMP3 knockdown cells are transfected with a plasmid to express Amyloid Precursor Protein

Express APP

The culture media is collected. Cells are scraped and lysed in 1% Triton X-100 lysis buffer with protease inhibitors

Collect Cells and Media

Total protein levels in lysates and media are quantitated using the Thermo Scientific™ Pierce™ BCA Protein Assay

Measure Total Protein Concentrations

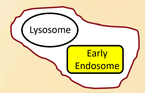
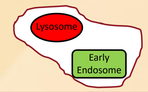
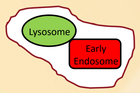
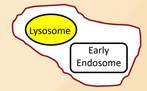
Quantitate and compare secreted and internal β–amyloid levels in control and SCAMP3 KD cells

Measure β-amyloid levels using an ELISA

http://www.enzolifesciences.com/

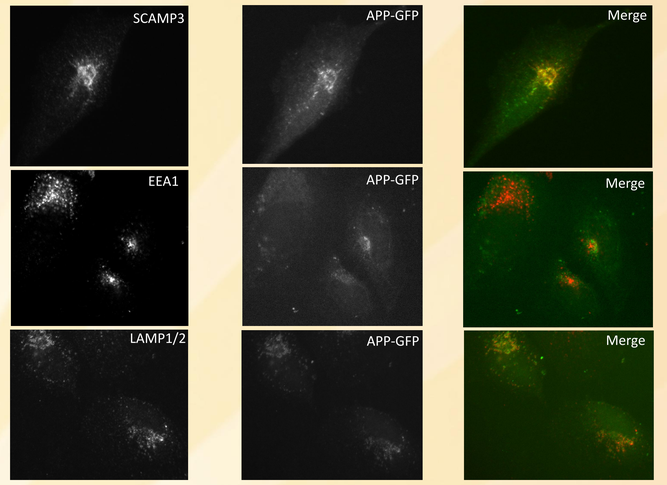
Potential Outcomes: Immunofluorescence Assay

In cells with a SCAMP3 knock-down, APP will be labeled with green fluorescent protein (GFP). The early endosomal marker, EEA1, or the lysosomal marker, LAMP1/2, will be labeled with a red fluorophore. Colocalization between APP-GFP and organelle markers will result in overlap of the red and green signals to produce yellow. If there is no colocalization, the red and green will appear separately.

Hypothesis	Effect of SCAMP3 Knock-down	Localization Relative to Early Endosome	Localization Relative to Lysosome
SCAMP3 promotes APP trafficking to the lysosome	APP trafficking to the lysosome will decrease		
SCAMP3 inhibits APP trafficking to the lysosome	APP trafficking to the lysosome will increase		

Results

Colocalization of transiently transfected APP with SCAMP3, EEA1, and LAMP1/2



The images above show three different cells, separated into different rows. The images were taken after culturing to allow expression of APP-GFP and either SCAMP3, EEA1, or LAMP1/2 (marked with red fluorophore). Images were taken to show expression of each marker, then colored and superimposed to show colocalization. From the merged images, there is colocalization of APP with SCAMP3, EEA1, and LAMP1/2 within normal H4 cells. However, the least colocalization is seen between APP and EEA1.

Conclusions and Next Steps

The results show that APP-GFP is colocalized with LAMP1/2 which fits our second hypothesis, that SCAMP3 inhibits trafficking of APP to the lysosome. This could imply that greater expression of SCAMP3 within neurons could lead to greater production of β-amyloid, and thus earlier or more severe onset of Alzheimer's Disease. More testing needs to be done, and with positive results the experiment could move on to testing in neuronal cells. If there are still significant results from there, the potential could be development of potential treatments for Alzheimer's Disease. Further confirmation of experimental results are needed by measuring β-amyloid levels with the ELISA assay.

References

[1] Aoh, Q. L., Castle, A. M., Hubbard, C.H, Katsumata, O., Castle, J. D. Mol Biol Cell. 2009 Mar; 20 (6); [2] Castle, A., and Castle, D. J Cell Sci. 2005 Aug 15 (16); [3]. Edgar, J. R., K. Willen, G. K. Gouras, and C. E. Futter. "ESCRTs Regulate Amyloid Precursor Protein Sorting in Multivesicular Bodies and Intracellular Amyloid- Accumulation." Journal of Cell Science 128.14 (2015): 2520-528. [4] Fernandez-Chacon R., Sudhof T. C. J Neurosci. 2000 Nov 1; 20 (21; [5]Hubbard, C., Singleton, D., Rauch, M., Jayasinghe, S., Cafison, D., Castle, D. Mol Biol Cell. 2000 Sep 11 (9) [6]. Raiborg, Camilla, and Harald Stenmark. "The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins." *Nature* 458.7237 (2009): 445-452. [7]. 2017 Enzo Life Sciences, Inc., http://www.enzolifesciences.com/ADI-EKS-446/nfkappab-p65-elisa-kit/