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Analysis of Proteolytic Cleavage of Fibril Forming Lysosomal Protein

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Summer Undergraduate Research in Biology

Abstract

Cytotoxic protein misfolding and aggregation play a causative role in age-related neurodegenerative diseases, including Alzheimer's, Huntington's, and Parkinson's diseases. Recent studies have revealed cytosolic aggregation of the TMEM106B protein to increase with age, to accumulate in frontal lobe dementia, and to potentially contribute to other neurodegenerative diseases (Schweighauser et al., 2022; Chang et al., 2022). The wild-type protein has been predicted to locate to the lysosomal membrane, while a proteolytic cleavage process results in a fragment of the protein locating to the cytosol (Schweighauser et al., 2022). It is known that the release of the C-terminal domain from the full length protein results in fibril formation *in vivo* studies (Schweighauser et al., 2022; Chang et al., 2022). In our study, we sought to establish a cell culture-based assay for the activation and downstream implications of TMEM106B cleavage. We hypothesized that virus-mediated expression of TMEM106B in cultured cell lines would result in localization of this protein to the lysosome and that lysosomal membrane permeabilization caused by Leu-Leu methyl ester (LLOMe) would cause TMEM106B to be cleaved by damage-induced proteolysis. Western blot analysis coupled with fluorescent microscopy indicates that lentiviral delivery of TMEM106B was successful in BHK-21 hamster fibroblasts; however, localization and cleavage are to be further explored. Neuronal SH-SY5Y cells are also being cultured following viral infection with TMEM106B and analysis is pending.

Introduction

TMEM106B is a 274 amino acid transmembrane lysosomal protein whose function is not yet fully characterized (Schweighauser et al., 2022). It is known to be expressed in neurons, glial cells, and endothelial cells. As shown in Figure 1, TMEM106B consists of an N-terminal domain that projects into the cellular cytosol, a single-pass transmembrane alpha helix, and a C-terminal domain that extends into the lysosomal lumen (Schweighauser et al., 2022). The lysosome is an organelle containing digestive enzymes and its main function is to break down worn out cellular components. Recent studies have suggested that some neurons displaying characteristics of neurodegenerative diseases have a cleaved version of TMEM106B (Chang et al., 2022). In fact, the cleaved protein was shown to form amyloid fibrils that are prevalent in neurodegenerative proteinopathies (Chang et al., 2022). The cleavage process is hypothesized to be carried out by lysosomal proteases, though the localization and the cleavage process are ongoing areas of experimental examination.

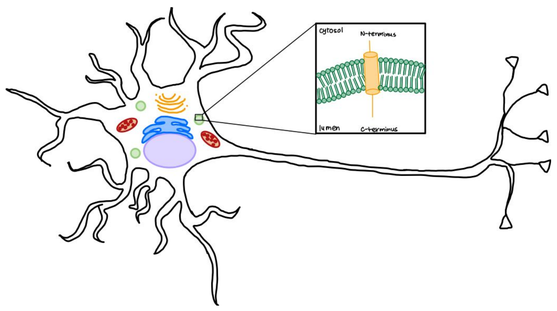

Figure 1: The structure and location of TMEM106B in a neuron.

This investigation aimed to use a lentiviral delivery of an expression construct to cultured cells, establish cellular localization of the expressed protein, and then use a lysosomal stressor to assess activation of TMEM106B cleavage. This was done in preliminarily BHK-21 cells with future research to work on SH-SY5Ys, a human neuronal cell line.

Results

Lentiviral Transduction

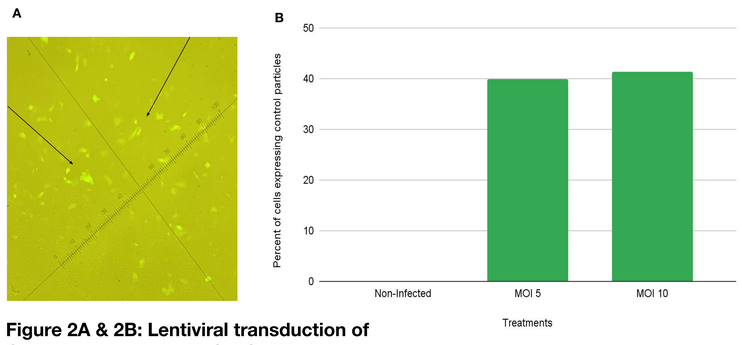

Figure 2A & 2B: Lentiviral transduction of GFP was successful in SH-SY5Y cells.
SH-SY5Ys were seeded at 25,000 cells per cm^2. GFP control lentiviral particles were used to infect the cells at an MOI of 5 and 10 with 10µL/mL polybrene. Media was changed 24 hours after lentiviral transduction. (A) Images were taken of SH-SY5Y cells expressing GFP. (B) The percentage of cells expressing GFP three weeks post transduction was quantified.

Cell Fractionation of Lentiviral Stable Myc/DDK-TMEM106B

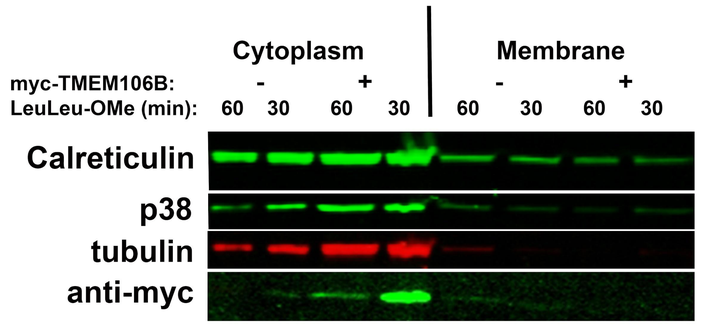

Figure 3: Immunoblot indicates successful lentiviral transduction of TMEM106B. Uninfected and TMEM106B infected (MOI 10) BHK-21s cells were seeded at 5 x 10^6 cells and let sit for 24 hours. A 0.1mM of LLOMe was added for 30 minutes and 60 minutes. Cells were suspended in DPBS, centrifuged, and were resuspended using a lysis buffer containing digitonin in order to disrupt the cellular membrane. The lysis buffer and pellet solution was incubated, centrifuged, and the supernatant was collected and labeled as the cytosolic fraction. A second lysis buffer, containing NP-40, was added to puncture membrane organelles, incubated, centrifuged, and supernatant was saved as the membrane portion. 40µL of cytosolic fractions with 10µL of loading buffer and 20µL of membrane fractions with 8µL of loading buffer were added to a 4-20% tris-glycine gel. The gel was transferred to a polyvinylidene fluoride (PVDF) and blocking took place using a 1:3 dilution of Odyssey Blocking Buffer. Primary antibodies Calreticulin, p38, tubulin, and myc were added at 10:20,000 dilutions and after TBST washing, fluorescent secondary antibodies were added at 1:20,000 dilution. Results indicate that cells were successfully infected with TMEM106B.

Fluorescent Microscopy of TMEM106B Infected Cells

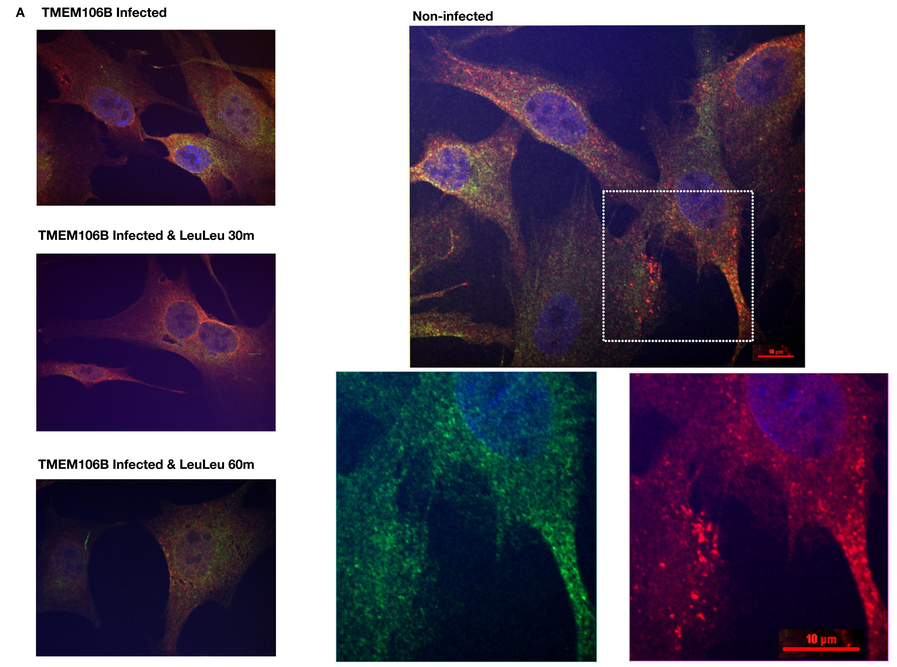

Figure 4A, 4B & 4C: Fluorescent microscopy shows colocalization of LAMP1 and myc tagged TMEM106B. BHK-21 cells were seeded at 15,000 cells/cm^2. TMEM106B cells were infected at an MOI of 10. Cells were fixed in 4% PFA. An anti-LAMP1 antibody and an anti-myc antibody were added at a 1:150 dilution. Cells were mounted onto slides with ProLong gold antifade reagent with DAPI to stain the nuclei blue. (A) Images were collected of uninfected, TMEM106B infected, and TMEM106B infected cells treated with LLOMe for 30 and 60 minutes. (B) LAMP1 tagged lysosomes show a decrease with LLOMe treatment. (C) Bright red puncta tagging LAMP1, a lysosomal protein, were detected with 0.55µm and a contrast of 195, while bright green puncta, tagging myc, were detected with 0.55µm and a contrast of 70 and overlap of puncta was quantified.

Conclusions

- Immortalized BHK-21 hamster fibroblasts can be infected with TMEM106B, suggesting it can be done in other immortalized cell lines
- C-terminal tagged TMEM106B can be identified through westerns and imaging
- Immortalized SH-SY5Y human neurons can be infected with GFP control particles, suggesting that they can also express TMEM106B

Works Cited

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Acknowledgements

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