Differential Expression of Cathepsin L in Alzheimer's Disease Brain Tissue and Neural Cell Lines

Braden C. Younglove[1], Alisha M. Harrison[2], Chandana K. Uppalapati[3], Elizabeth E. Hull[2] and Kathryn J. Leyva[3]

[1]Arizona College of Osteopathic Medicine, [2]Biomedical Sciences Program, & [3]Department of Microbiology & Immunology Midwestern University, Glendale AZ

Abstract

Background: Progranulin (PGRN) and cathepsin L (CTSL) are linked to the development of several neurodegenerative diseases (NDD), including AD. CTSL mRNA splice variants have been identified, but there is no information on their relationship to disease pathology in NDD. Our hypothesis is that CTSL protein expression levels and/or mRNA variants are produced in response to inflammatory stimuli, which may alter lysosomal function and affect PGRN processing, resulting in AD pathology. PGRN is processed into functional granulin units by proteases, including CTSL, and is active at acidic & neutral pH. Mutations in *CTSL* or aberrant processing of CTSL cause altered lysosomal function that contributes to NDD. However, the link between PGRN and CTSL in regulating lysosomal function and inflammation is unclear. There are ~10 different CTSL mRNA variants characterized, yet there is little information on the significance of variants to disease pathology in NDD. Thus, characterization of mRNA splice variants may clarify their individual functions, facilitating the development of therapeutics aimed at restoring lysosome function.

Methods: Immunoblotting and immunohistochemistry using anti-CTSL antibodies were performed on cell lysates and fixed brain tissues, respectively, from normal and AD patients to determine CTSL expression. Anti-p-Tau was used to determine co-localization with CTSL. Assessment of CTSL mRNA variants was done using a microglial (HMC3) and neuronal (SH-SY5Y) cell line. Cells were cultured in the presence of pro- or anti-inflammatory cytokines, with untreated cells as a control. RT-PCR was performed using combinations of specific primers to identify the expression of CTSL mRNA variants, and the results were analyzed using gel electrophoresis. RT-PCR was also performed on AD and normal patient tissue samples. CTSL activity was measured using a fluorometric assay on cell lysates from cytokine-treated and untreated HMC3 and SH-SY5Y cells.

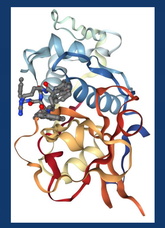
Results: Immunoblot and IHC results show CTSL protein expression is higher in AD patient samples than in normal tissues, and CTSL appears to be co-localized with p-Tau. PCR analysis reveals that both cell type and cytokine treatment yielded differential CTSL mRNA splicing. CTSL activity of HMC3, but not SH-SY5Y, cells was higher when treated with a proinflammatory cytokine; interestingly, CTSL activity was higher in SH-SY5Y, but not HMC3, cells when treated with anti-inflammatory cytokines. We also observed differential expression of CSTL mRNA variants between AD brain, AD thalamus, and normal tissues.

Discussion/Conclusions: Our data suggest that differential expression of CTSL, which may impact CTSL and PGRN activity, is occurring in the context of inflammation. Future research will focus on sequencing mRNA variants to determine any correlation between CTSL variant(s), inflammation, and protease activity, which may correlate to AD pathology. Our results will lay the groundwork for determining if CTSL expression and/or activity can be controlled by differential RNA processing, and if so, may provide additional avenues to explore for the development of therapeutics that target early stages of AD or other NDDs.

Introduction

❖ **Cathepsin L (CTSL)** is a lysosomal cysteine protease that plays a critical role in maintaining neuronal health. Beyond its role in protein degradation, CTSL regulates key cellular processes, including axonal & myelin turnover, neuropeptide processing, and microglial inflammatory responses. Dysregulation of CTSL expression or activity has been linked to neuroinflammation, neuronal damage, and abnormal processing of progranulin (PGRN)—a lysosomal protein associated with cognitive decline. Importantly, elevated CTSL has been observed in neurons and amyloid plaques in AD brains, while inhibition of CTSL can reduce microglia-driven neuroinflammation.

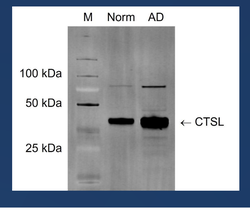
CTSL

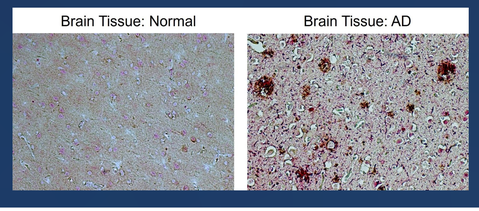


https://www.sinobiological.com/resource/cathepsin-l

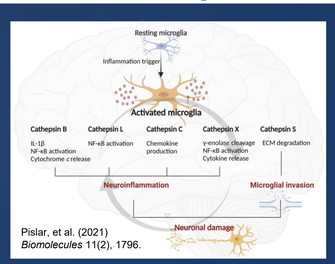
Immunoblotting & Immunohistochemistry

Brain Tissue from AD Patients Have Higher Levels of CTSL. Immunoblotting (left) shows increased CTSL in AD brain tissue. IHC (right) using anti-CTSL and anti-pTau antibodies also show higher levels of CTSL with CTSL co-localizing with p-Tau in AD brain tissue. Similar pathologic changes have been observed in Lewy body dementia [1].





Cathepsin L & Neuroinflammation



Pislar, et al. (2021) *Biomolecules* 11(2), 1796.

Cathepsin Activity is Involved in Neuro-inflammation, Resulting in Damage [2].

❖ **Our Hypothesis**: CTSL protein expression levels and/or mRNA variants are produced in response to inflammatory stimuli, which may alter lysosomal function and affect PGRN processing, resulting in pathology associated with Alzheimer Disease (AD).

Cathepsin L Primer Design

Cathepsin L Primer Visual - [87,726,119..87,731,469]

Cathepsin L

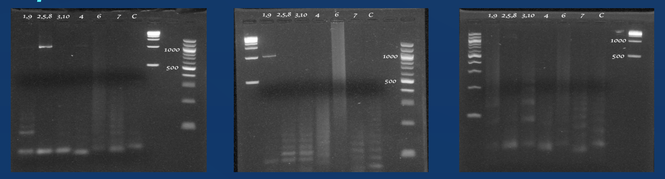
nt	1	100	150	200	250	300	350	400	1100	1200	1300	1400	1500	1644
1+9					1,9 F					1,9R				
3+10			3,10 F						3,10R					
2+5+8		2,5,8 F							2,5,8R					
4		4 F							4R					
6						6 F		6R						
7		7 F												
C									C F			C R		

	CTSL	CTSL 1,9	CTSL 3,10	CTSL 2,5,8	CTSL 4	CTSL 6	CTSL 7	CTSL C
Forward	1-1644	267-286 nt	176-196 nt	121-140 nt	118-137 nt	337-356nt	124-143 nt	1125-1144 nt
Reverse	N/A	1246-1227 nt	1158-1139 nt	1101 - 1082 nt	1097-1078 nt	443-424 nt	856-837 nt	1440 - 1421 nt

Primer Design to Identify Cathepsin L Variants: Primers were designed to selectively amplify splice variants of cathepsin L, ensuring specificity for regions of interest while minimizing off-target amplification.

Results

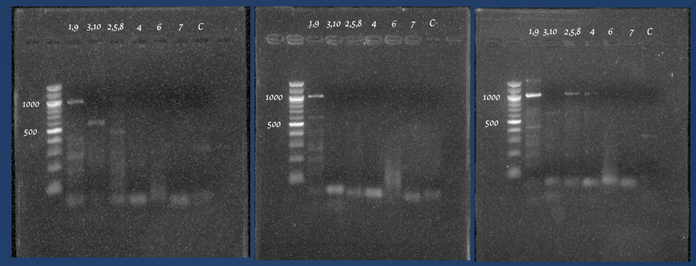
Cathepsin L Variants Identified in Tissues from AD Patients



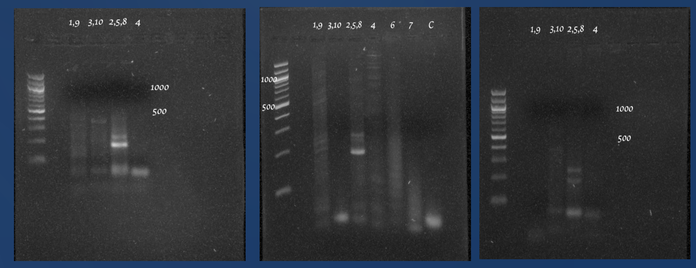
Cathepsin L Variants Identified in AD Patient Tissues: PCR was performed on extracts from thalamus brain tissue (left) and cortex brain tissue (center) from Alzheimer patients for CTSL variants; PCR performed on extracts from normal tissue is shown (right). Diseased brain tissue shows presence of cathepsin L splice variants compared to normal brain tissue. We observed a different CTSL splice variant in the thalamus compared to the cortex in AD patients, neither of which were observed in extracts from normal patient brain tissue.

Results

Cathepsin L Variants in HMC3 Microglial & SH-SY5Y Neuronal Cells

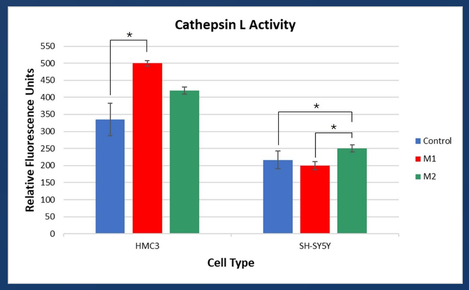


PCR for CTSL Variants in HMC3 Microglial Cells. HMC3 cells were either untreated (left), treated with anti-inflammatory (M2) cytokines (middle), or treated with a pro-inflammatory (M1) cytokine (right). CTSL splice variants were noted among the treatments.



PCR for CTSL Variants in SH-SY5Y Neuronal Cells. SH-SY5Y cells were either untreated (left), treated with anti-inflammatory (M2) cytokines (middle), or treated with a pro-inflammatory (M1) cytokine (right). Cytokine treatment resulted in expression of a CTSL variant not observed in untreated cells.

Difference in Cathepsin L Protease Activity



Cytokine Treatment Alters Cathepsin L Activity: HMC3 microglial cells were treated with a pro-inflammatory (M1) or a mix of anti-inflammatory (M2) cytokines and assessed for cathepsin L activity using a fluorometric assay kit (AnaSpec). Overall, cathepsin L activity was higher in the HMC3 microglial cell line compared to the SH-SY5Y neuronal cell line. For HMC3 cells, treatment with a pro-inflammatory (M1) cytokine resulted in higher CTSL activity compared to control. For SH-SY5Y cells, treatment with ant-inflammatory (M2) cytokines resulted in higher CTSL activity compared to untreated and M1-treated cells.

❖ **Next Steps:** Sequence each PCR product to determine if there are differences in the cathepsin L sequences among the different treatments and region of the brain.

Acknowledgements

Funding: Arizona Alzheimer Consortium Pilot Grant and Midwestern Arizona Alzheimer's Consortium (MAAC) Grant awarded to EEH and KJL. MWU provided intramural grant funds to EEH and KJL. BCY was supported by a MWU Kenneth A. Suarez Research Fellowship.

References

1. Balin BJ et al. Intriguing mixed pathologic features in a case of dementia with Lewy bodies. *J Osteopath Med*. 2019;119(9):632–636. doi:10.7556/jaoa.2019.106
2. Pislar, AH et al. New insights into the role of cysteine cathepsins in neuroinflammation. *Biomolecules* 2021; 11(12):1796. https://doi.org/10.3390/biom11121796