

TITLE: Changes in expression of genes associated with autophagy and apoptosis in neuronal cells infected with HSV-1 may suggest infection-induced mechanisms of neurodegeneration

AUTHORS: Alexis Mark¹, Fiora D. Zoga¹, Brian J. Balin^{1*}, Denah M. Appelt^{2*}, Susan T. Hingley^{1*}

AFFILIATIONS: Department of ¹Pathology, Microbiology, Immunology and Forensic Medicine and ²Neuroscience, Pharmacology, and Physiology and *Center for Chronic Disorders of Aging, Philadelphia College of Osteopathic Medicine

ABSTRACT:

Background

This study investigates the potential role of herpes simplex virus type 1 (HSV-1) in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD), by exploring changes in gene expression related to antiviral immunity and the autophagic pathway. Autophagy is a process that recycles organelles and proteins to create more energy for the cell. This pathway has been linked to neurodegeneration, as malfunctions in the completion of this process lead to a decline in overall cellular health and function. Interestingly, HSV-1 has been shown to block the completion of autophagy, which would potentially contribute to the cytopathic changes observed in AD.

Materials & Methods

Antiviral and autophagy reverse transcriptase, real time PCR (RT²-PCR) microarrays were used to analyze changes in gene expression in HSV-1-infected SK-N-MC human neuronal cells relative to uninfected cells. Immunofluorescent labeling for lysosomal associated membrane protein-1 (LAMP-1) and an autophagosome marker, protein 1 light chain 3 (LC3B), was used to determine whether lysosomes and autophagosomes colocalized in SK-N-MC cells after being infected with HSV-1. Labeling of LAMP-1 and LC3B in HSV-1-treated cells was compared to that in cells treated with rapamycin (RM) or chloroquine (CQ), known activators of autophagy.

Results

Genes exhibiting greater than a 2.5-fold increase in mRNA levels in HSV-1-infected SK-N-MC cells relative to levels in uninfected cells include genes encoding chemokine ligands (CXCL10, CXCL11, CXCL9) and various cytokines (IFN α 2, IFN α 4, IFN γ , IL1 β , IL8, IL15 and TNF). In addition, numerous genes associated with autophagy regulation (CASP8, CXCR4, FAS, PIK3CG), and autophagosome or autolysosome formation (ATG9B, DRAM1, FAM176A) are upregulated. HSV-infected SK-N-MC cells, as well as RM- or CQ-treated cells, demonstrated an increase in labeling for LC3B as compared to uninfected and untreated cells, indicating that autophagy was induced. Lysosome formation, seen as an increase in labeling for LAMP, was also increased in infected or treated neuronal cells relative to untreated/uninfected cells, especially following treatment with CQ.

Conclusions

Data reported here demonstrate HSV-1-mediated activation of autophagy within SK-N-MC cells. Furthermore, immunofluorescence labeling indicates that while autophagosomes and lysosomes are more prevalent in HSV-1-infected SK-N-MC cells, there is little or no colocalization of the

two structures. This observation suggests that while autophagy initiation has taken place, the autophagic pathway may not go to completion. Further analysis of the impact of HSV-1 on autophagy may suggest a mechanism by which HSV-1 can contribute to some of the pathological changes in neurons associated with neurodegeneration.